Memoirs of the Department of Agriculture in India

VETERINARY SERIES

Vol. III

Agricultural Research Institute, Pusa

PUBLISHED FOR
THE IMPERIAL DEPARTMENT OF AGRICULTURE IN INDIA

Calcutta: Government of India
Central Publication Branch
1927

EDITED BY

The Council of the Pusa Agricultural Research Institute which is not, as a body, responsible for the opinions expressed in the Memoirs.

CONTENTS

VOL III

CONTENTS.

THE VITALITY OF THE RINDERPEST VIRUS OUTSIDE THE ANIMAL BODY UNDER NATURAL CONDITIONS.

BY

A. W. SHILSTON, M.R.C.V.S.,

Assistant Bacteriologist, Muktesar Laboratory.

[Received for publication on the 17th January, 1917.]

I. INTRODUCTION.

Although rinderpest has been the subject of extensive investigations in many countries, great uncertainty has existed in regard to the length of time that the virus is able to survive outside the animal body either under natural conditions or when special measures are taken to preserve its vitality. The question of the duration of rinderpest infection apart from affected animals is, however, of great importance in relation to the control of the disease in the field, and the observations recorded in this Memoir were carried out with a view to obtaining exact information on this point under varying conditions in India.

Reference to the literature on the disease shows the extent to which the views of various authorities differ.

Law[1] quotes Müller and Dieckerhoff as stating that "on stalls, mangers and racks in an obscure and still atmosphere, virulence may be preserved for three months" and Chauveau as stating that "in litter and manure in the open air, and even in yards and pastures it may retain its vitality for weeks."

Friedberger and Fröhner[2] state that "Nasal mucus hermetically sealed up will remain virulent for six weeks and in a few cases even for nine months. The infective matter remains active in sheds for four months and in hay for as long as five months. Flesh retains its virulence after it has been buried for

[1] Law, J. *Veterinary Medicine*, 2nd edition, vol. IV, p. 676.

[2] Friedberger & Fröhner. *Veterinary Pathology*, 6th edition, English Translation, vol. II, p. 625.

three months and manure which continued in a frozen condition throughou the winter proved infective after it was dug up in the spring."

Williams and Baldrey[1] quote Prof. Jessen of Dorpat as saying that "the mucus discharges, carefully protected, occasionally retain their power of causing the disease by inoculation for no less than eleven months."

Ruediger[2] states: "Pastures that have been infected by sick animals may remain infected for months or even for years."

Hutyra and Marek[3] give the following references:—

"According to older observations (Haubner, Dieckerhoff) the virus remains virulent on hay kept in the stable or in the hay loft for 3 to 4 months, but when exposed to the sunlight it is destroyed in two days.

"Meat which has been buried for three months is supposed to have been still infective (Vicq D'Azyr).

"Arloing, however, found that the virus retains its virulence in meat only four days. The infectiveness of manure, which has been contaminated with the excrements of affected animals, contains the virus for one month according to Bouley and according to Krajewski it may sometimes be infective even after three months. More recent investigations in this direction proved however that the virus is destroyed by putrefaction in a few days (Kolle)."

Hutcheon[4] states that complete desiccation destroys the virus.

Edington[5] states: "Blood can retain its virulence when quickly dried in a very thin layer, but if it is dried in a thick layer its virulence is destroyed."

Stockman[6] states that virulent material does not remain active for more than a day or two outside the animal body.

Refik Bey and Refik Bey[7] state: "Infected areas do not remain dangerous for long if we may believe our own observations. We regard the rinderpest

[1] Williams. *Veterinary Medicine*, W. O. Williams and F. S. H. Baldrey, 9th edition, p. 150.

[2] Ruediger, E. H. Observations on Cattle Plague in the Philippines. *Phil. Journal of Science*, Sec. B., 1909, no. 4, p. 381.

[3] Hutyra & Marek. *Special Pathology of the Diseases of Domestic Animals*, translation by Mohler & Eichhorn, 1912, vol. I, p. 237.

[4] Hutcheon. *Journal of Comparative Pathology*, vol. XV, 1902, p. 300.

[5] Edington. *Lancet*, vol. I, 1899, p. 338.

[6] Stockman, S. Note on the Methods of Combating Rinderpest. *Journal of Comp. Path.*, vol. XVIII, 1905, p. 208.

[7] Refik Bey & Refik Bey. La peste bovine en Turquie. *Ann. Inst. Pasteur*, vol. XIII, 1899, no. 7, p. 600.

virus as essentially fragile and incapable of development in external media."

Ward, Wood, and Boynton[1] carried out several experiments in the Philippine Islands to test the duration of rinderpest infection under natural conditions and came to the following conclusions :—

(1) Rinderpest virus was not shown to have survived beyond twenty-four hours in corrals bare of vegetation but containing water.

(2) Animals became infected in such corrals within half-an-hour, twelve hours and seventeen and one-half hours respectively after removal of the sick.

(3) The virus in urine diluted with water and sprinkled on grass was demonstrated to survive for thirty-six hours in some instances but not always and not for a longer period of time.

(4) Fæces mixed with water and sprinkled on grass infected an animal twenty-four hours later.

(5) Fæces and urine diluted with water and kept in a vessel in the shade remained infective for susceptible animals for thirty-six hours but no longer.

(6) The foregoing facts indicate that the virus of rinderpest perishes soon after being discharged by the infected animal.

(7) Nothing in the foregoing experiments indicates that rinderpest virus is harboured for long periods upon the soil of contaminated areas.

The observations recorded in the present Memoir were designed to ascertain the duration of the vitality of the rinderpest virus (i) on ground in the open air, (ii) in closed sheds, (iii) in fæces, urine and mucos discharges, and (iv) in meat, blood, and bones under varying natural conditions.

In order that the tests might be as complete as possible they were carried out both at the Muktesar Laboratory, 7,500 feet above sea-level, and, with the exception of the tests of meat, blood, and bones, at Bareilly under plains conditions. The experiments in each case will be described separately.

[1] Ward, A. R., Wood, F. W., and Boynton, H. Experiments upon the Transmission of Rinderpest. *The Phil. Journal of Science B.*, vol. IX, 1914, p. 77.

II. DURATION OF RINDERPEST INFECTION ON GROUND IN THE OPEN.

(a) Observations at Muktesar.

Several authorities who state their opinion that rinderpest infection may persist, under certain conditions, for long periods refer to the rapidity with which the virus is destroyed by exposure to sunlight.

To determine therefore to what extent the duration of ground infection was influenced by direct sunlight, two small wire enclosures were constructed, one without any protection against the sun and the other almost entirely shaded by overhanging trees. (Plates I and II).

For the purpose of infecting the enclosures, two hill bulls suffering from acute rinderpest, with vesicles in the mouth and diarrhœa, were turned into each and allowed to remain until in a dying condition. They were then removed and at varying intervals healthy hill bulls were placed in the enclosures to test the existence of infection. Each test animal was kept in the enclosure at least 24 hours, usually longer, and was afterwards disinfected and segregated with healthy hill bulls to control the possibility of subsequent outside infection.

In the case of those animals which contracted the disease, the diagnosis was confirmed by *post-mortem* examination or blood inoculation. The susceptibility of all animals which failed to react was subsequently tested by the inoculation of virulent blood or, in a few cases, exposure to infection in later experiments.

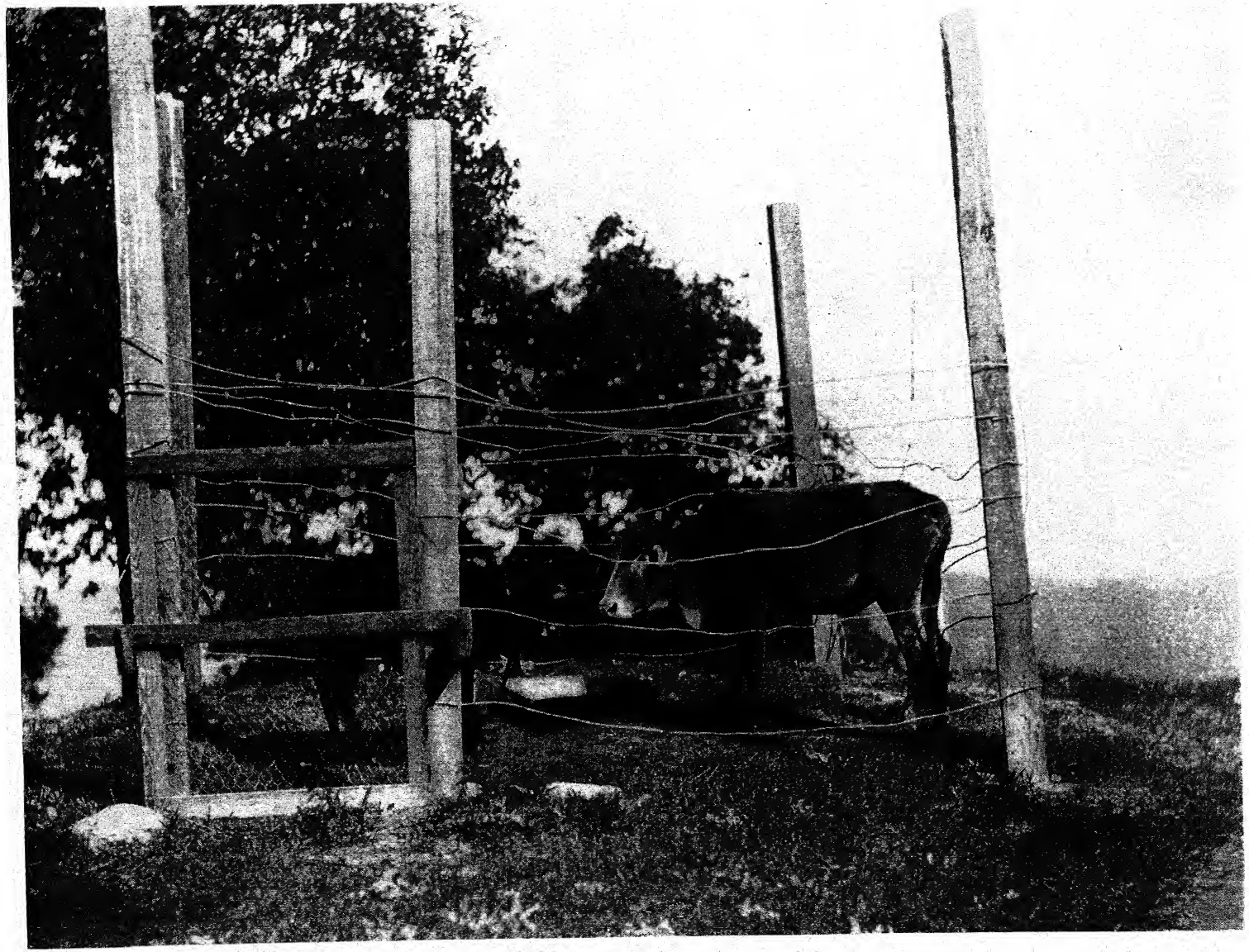
During the period covered by the tests all varieties of weather condition were experienced, except great cold. The daily maximum shade temperature varied from 63° to 82° F. and the minimum from 47° to 62° F.

The following are the details of the observations made in the two enclosures :—

(1) *The duration of rinderpest infection in the enclosure without shade.*

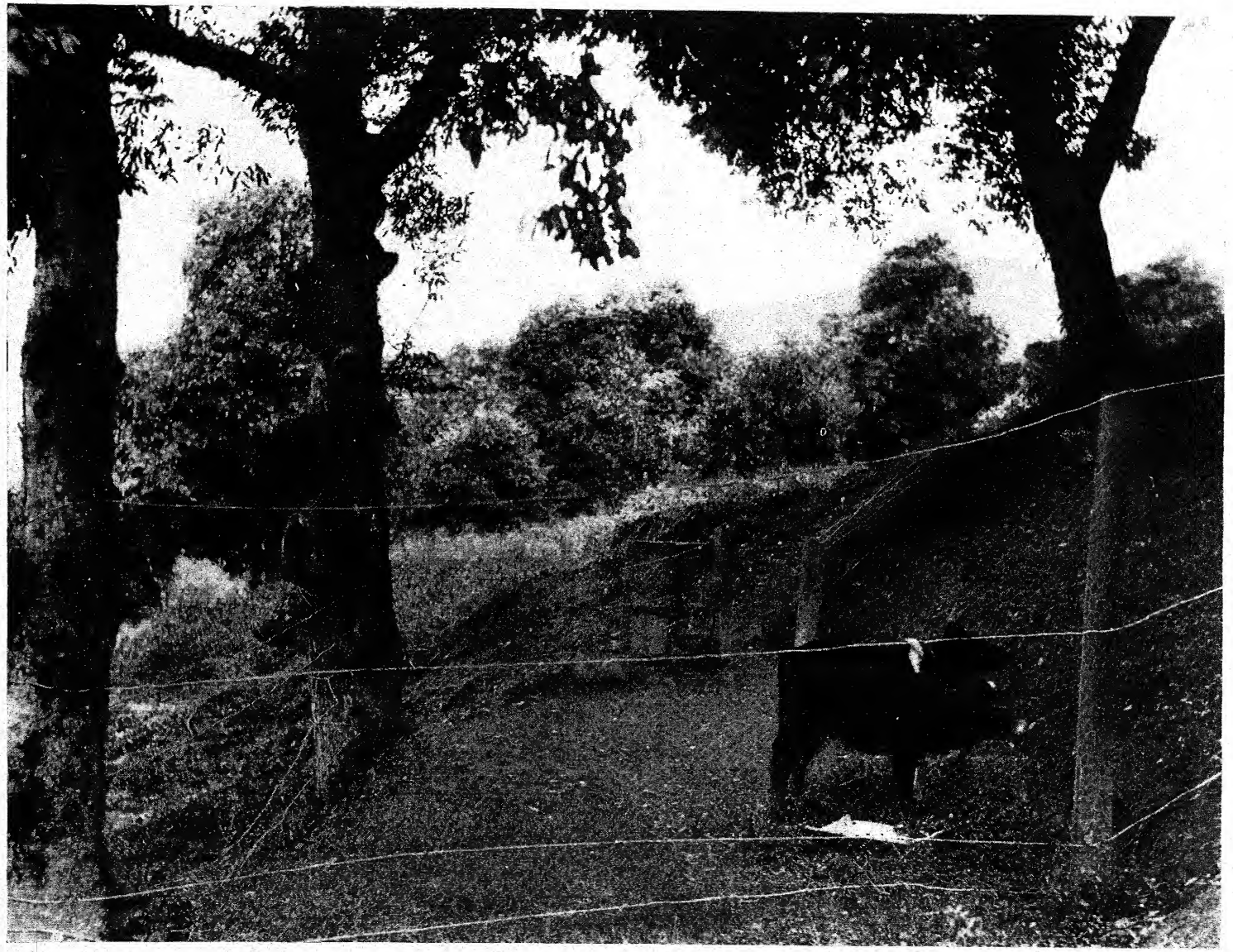
Experiment 1.

1916, May 19th	..	.. Hill bulls 6797 & 6798, fourth day rinderpest attack, placed in the enclosure to infect it.
„ 23rd, 11 A.M.	..	.. Sick animals 6797 & 6798 removed. Bright sunshine.



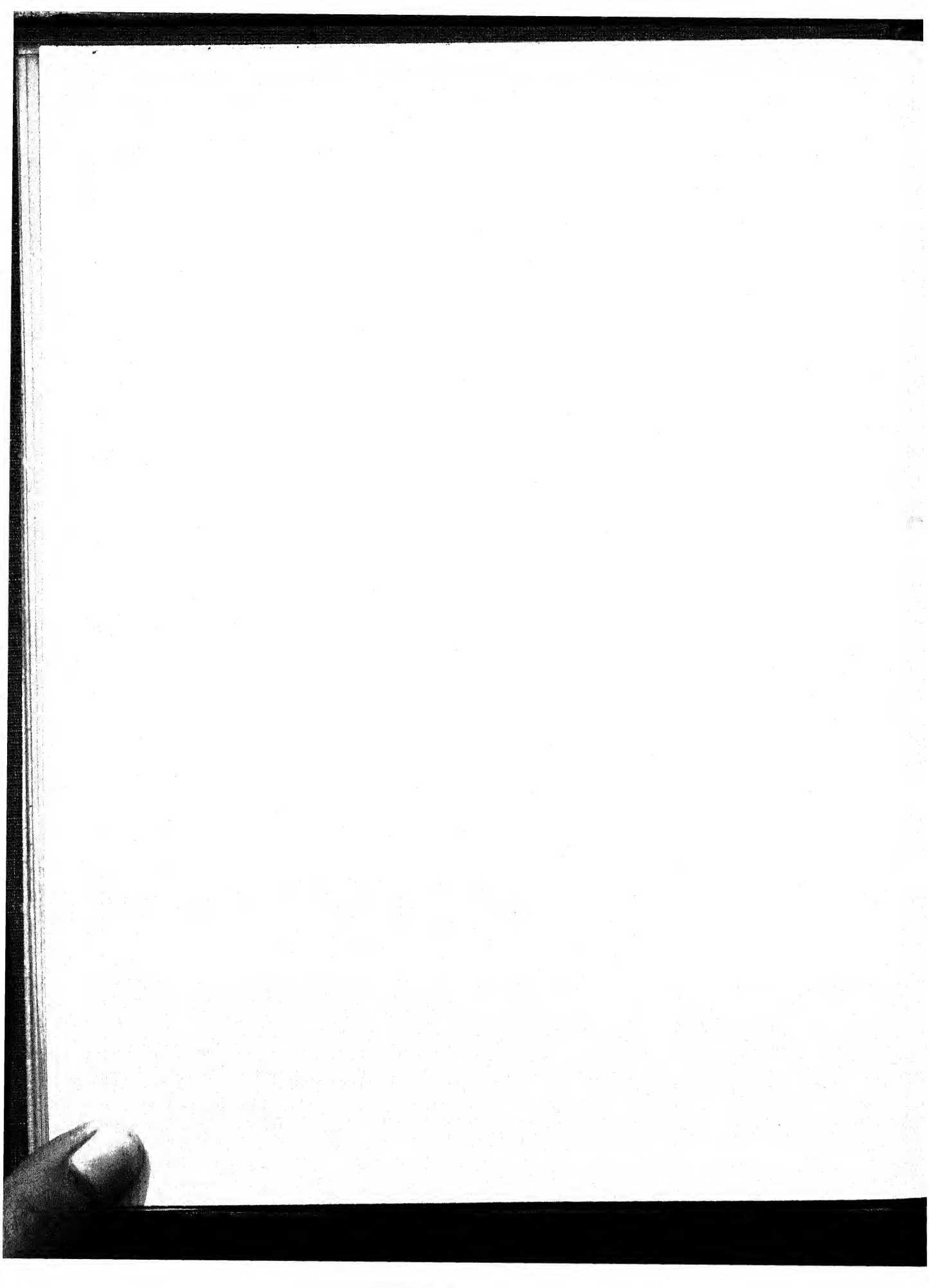
THE ENCLOSURE WITHOUT SHADE USED IN THE TESTS AT MUKTESAR.

PLATE



THE ENCLOSURE SHADED BY TREES USED IN THE TESTS AT MUKTESAR.

PLATE II.



1916, May 24th, 11 A.M. ..	.. Hill bull 6813 placed in the enclosure. Bright sunshine.
„ 25th, „ „ ..	.. Hill bull 6816 placed in the enclosure. Bright sunshine.
„ 26th, „ „ ..	.. Hill bull 6813 removed. Bright sunshine.
„ 30th ..	.. Hill bull 6816 removed. Bright sunshine.

Result. Neither test animal contracted rinderpest, though both were susceptible. Infection not present 24 hours after removal of the sick animals.

EXPERIMENT 2.

1916, June 4th	.. Hill bulls 6826 and 6827, fifth day rinderpest attack, placed in the enclosure.
„ 6th, 4 P.M. ..	.. Hill bulls 6826 and 6827 removed. Bright sunshine.
„ 7th, 8 A.M. (16 hours)	.. Hill bull 6813 placed in the enclosure. Dull and misty.
„ „ 12 noon (20 „)	.. Hill bull 6835 placed in the enclosure. Dull and misty.
„ 8th, 4 P.M. ..	.. Both animals removed. Sun and rain at intervals.

Result. Neither test animal contracted rinderpest though both were susceptible. Infection not present after 16 hours.

EXPERIMENT 3.

1916, Aug. 2nd, 11 A.M. ..	.. Hill bulls 6980 and 6982, sixth day of rinderpest attack, placed in the enclosure.
„ 3rd, 4 P.M. ..	.. Sick bulls 6980 and 6982 removed. Sky cloudy.
„ 4th, 8 A.M. (16 hours)	.. Hill bull 7011 placed in the enclosure. Clouds and rain.
„ „ 12 noon (20 „)	.. Hill bull 7012 placed in the enclosure. Clouds and rain.
„ „ 4 P.M. (24 „)	.. Hill bull 7013 placed in the enclosure. Clouds and rain.
„ 5th, 4 P.M. ..	.. Test animals removed. Sky cloudy.

Result. The test animals did not contract rinderpest ; all were susceptible. Infection not present after 16 hours.

EXPERIMENT 4.

1916, Aug. 14th, 11 A.M. ..	.. Hill bulls 7030 and 7031, sixth day of rinderpest attack, placed in the enclosure. Rain at night, sun and clouds at intervals.
„ 15th, 7-30 A.M. ..	.. Sick bulls 7030 and 7031 removed. Sun and clouds at intervals.
„ „ 7-30 P.M. (12 hours)	.. Hill bull 7014 placed in the enclosure. Sun and clouds at intervals.
„ 16th, 7-30 A.M. (24 „)	.. Hill bull 7020 placed in the enclosure. Sky clear.
„ „ 1-30 P.M. (30 „)	.. Hill bull 7023 placed in the enclosure. Sky clear.
„ 17th, 7-30 A.M. ..	.. Hill bull 7014 removed. Sky clear.
„ „ 1-30 P.M. ..	.. Hill bulls 7020 and 7023 removed. Sky clear.

Result. The test animals did not contract rinderpest ; all were susceptible. Infection not present after 12 hours.

Experiment 5.

1916, Sept.	27th, 11 A.M. ..	.. Hill bulls 7278 and 7279, fourth day of rinderpest attack, placed in the enclosure. Sky clear.
„	29th, 10-30 A.M. ..	.. Sick bulls removed. Sun and clouds at intervals.
„	„ 3-30 P.M. (5 hours)	.. Hill bull 7309 placed in the enclosure. Sun and clouds at intervals.
„	„ 6-30 P.M. (8 „)	.. Hill bull 7310 placed in the enclosure.
„	30th, 6-30 A.M. (20 „)	.. Hill bull 7026 placed in the enclosure. Sun and clouds at intervals.
Oct.	1st, 10-30 A.M. ..	.. Test animals removed. Sun and clouds at intervals.

Result. The test animals did not contract rinderpest ; all were susceptible. Infection not present after 5 hours.

Experiment 6.

1916, Oct.	5th, 10-30 A.M. ..	.. Hill bulls 7314 and 7315, fifth day of rinderpest attack, placed in the enclosure. Sky clear.
„	7th, 9-30 A.M. ..	.. Sick bulls removed. Sun and clouds at intervals.
„	„ 2-30 P.M. (5 hours)	.. Hill bull 7353 placed in the enclosure. Sun and clouds at intervals.
„	„ 5-30 P.M. (8 „)	.. Hill bull 7352 placed in the enclosure. Sun and clouds at intervals.
„	8th, 5-30 A.M. (20 „)	.. Hill bull 7351 placed in the enclosure. Sun and clouds at intervals.
„	12th ..	.. Test animals removed.

Result. Hill bulls 7353 and 7352 contracted rinderpest, their temperatures rising on 5th day after exposure, vesicles 7th day. 7351 did not become infected but later was found to be susceptible. Infection present 5 and 8 hours after removal of the sick animals but not after 20 hours.

Experiment 7.

1916, Oct.	17th, 2 P.M. ..	.. Hill bulls 7370 and 7371, fourth day of rinderpest attack, placed in the enclosure. Sky clear.
„	20th, 10-15 A.M. ..	.. Sick bulls removed. Sky clear.
„	„ 3-15 P.M. (5 hours)	.. Hill bull 7404 placed in the enclosure. Sky clear.
„	„ 6-15 P.M. (8 „)	.. Hill bull 7405 placed in the enclosure. Sky clear.
„	21st, 6-15 A.M. (20 „)	.. Hill bull 7365 placed in the enclosure. Sky clear.
„	27th ..	.. Test animals removed.

Result. The test animals failed to contract rinderpest ; all were susceptible. Infection not present after 5 hours.

EXPERIMENT 8.

1916, Oct. 31st	..	..	Hill bulls 7448 and 7449, fifth day of rinderpest attack, placed in the enclosure. Sky clear.
Nov. 2nd, 10-30 A.M.	..	..	Sick bulls removed. Sky clear.
,, ,, 2-30 P.M.	(4 hours)	..	Hill bull 7464 placed in the enclosure. Sky clear.
,, ,, 6-30 P.M.	(8 ,,)	..	Hill bull 7465 placed in the enclosure. Sky clear.
,, 3rd, 6-30 A.M.	(20 ,,)	..	Hill bull 7466 placed in the enclosure. Sky clear.
,, 18th	..	..	Test animals removed.

Result. The test animals failed to contract rinderpest; all were susceptible. Infection absent after 4 hours.

(2) *The duration of rinderpest infection in the enclosure shaded by trees.*

EXPERIMENT 1.

1916, Aug. 2nd	..	..	Buffaloes 3265 and 3266, sixth day of rinderpest attack, placed in the enclosure. Sun and clouds at intervals, some rain.
,, 4th, 11 A.M.	..	..	Sick buffaloes removed. Sky cloudy.
,, 6th, 11 A.M.	..	..	Hill bull 6988 placed in the enclosure. Dull, rain at intervals.
,, 7th, 11 A.M.	..	..	Hill bull 7026 placed in the enclosure. Dull, rain at intervals.
,, 8th, 11 A.M.	..	..	Hill bull 7023 placed in the enclosure. Dull, rain at intervals.
,, 20th	..	..	Test animals removed.

Result. The test animals failed to contract rinderpest; all were susceptible. Infection absent after two days.

EXPERIMENT 2.

1916, Aug. 16th	..	..	Hill bulls 7015 and 7016, fourth day of rinderpest attack, placed in the enclosure. Sun and clouds at intervals.
,, 18th, 10-30 A.M.	..	..	Sick bulls removed. Sun and clouds at intervals.
,, 20th, 10-30 A.M.	(44 hours)	..	Hill bull 7033 placed in the enclosure. Sun and clouds at intervals.
,, ,, 6-30 P.M.	(56 ,,)	..	Hill bull 7026 placed in the enclosure. Rain and clouds at intervals.
,, 21st, 10-30 A.M.	(72 ,,)	..	Hill bull 7081 placed in the enclosure. Sky clear.
,, ,, 12 noon	..	..	Hill bull 7033 removed from the enclosure.
,, 23rd	..	..	Hill bulls 7026 and 7081 removed from the enclosure.

Result. The test animals failed to contract rinderpest; all were susceptible. Infection absent after 44 hours.

Experiment 3.

1916, Aug. 30th	..	..	Hill bulls 7104 and 7108, fifth day of rinderpest attack, placed in the enclosure. Dull, some rain.
Sept. 1st, 12 noon	..	..	Sick bulls removed. Sun and clouds at intervals.
„ 2nd, 8 P.M.	(32 hours)	..	Hill bull 7026 placed in the enclosure. Sky clear.
„ 3rd, 12 noon	(48 „)	..	Hill bull 7173 placed in the enclosure. Sky clear.
„ „ 8 P.M.	(56 „)	..	Hill bull 7174 placed in the enclosure. Sky clear.
„ 4th, 8 P.M.	..	..	Hill bull 7026 removed from the enclosure.
„ 5th, 8 P.M.	..	..	Hill bulls 7173 and 7174 removed from the enclosure.

Result. The test animals failed to contract rinderpest; all were susceptible. Infection absent after 32 hours.

Experiment 4.

1916, Sept. 29th	..	..	Hill bulls 7280 and 7281, fifth day of rinderpest attack, placed in the enclosure. Sun and clouds at intervals.
Oct. 1st, 12 noon	..	..	Sick bulls removed. Sun and clouds at intervals.
„ „ 6 P.M.	(6 hours)	..	Hill bull 7323 placed in the enclosure. Sun and clouds at intervals. Heavy rain at night.
„ 2nd, 6 A.M.	(18 „)	..	Hill bull 7324 placed in the enclosure. Sky dull.
„ „ 6 P.M.	(30 „)	..	Hill bull 7326 placed in the enclosure. Rain in the night.
„ 3rd, 12 noon	(48 „)	..	Hill bull 7332 placed in the enclosure. Dull, raining.
„ 6th	..	..	Hill bull 7323 removed from the enclosure.
„ 13th	..	..	Hill bulls 7324, 7326, and 7332 removed.

Result. Hill bull 7323 contracted rinderpest, temperature rising 5th day, vesicles 7th day. The remaining test animals failed to become infected though all were susceptible. Infection was present 6 hours after removal of the sick animals but not after 18 hours. Heavy rain fell between these intervals.

Experiment 5.

1916, Oct. 10th	..	..	Hill bulls 7338 and 7339, fifth day of rinderpest attack, placed in the enclosure. Sky clear.
„ 12th, 12 noon	..	..	Sick bulls removed. Sky clear.
„ „ 6 P.M.	(6 hours)	..	Hill bull 7368 placed in the enclosure. Sky clear.
„ 13th, 6 A.M.	(18 „)	..	Hill bull 7369 placed in the enclosure. Sun and clouds at intervals.
„ „ 6 P.M.	(30 „)	..	Hill bull 7374 placed in the enclosure. Sun and clouds at intervals.
„ 14th, 12 noon	(48 „)	..	Hill bull 7377 placed in the enclosure. Sky clear.

1916, Oct. 17th	..	.. Hill bulls 7368 and 7369 removed from the enclosure.
,, 24th	..	.. Hill bulls 7374 and 7377 removed from the enclosure.

Result. Hill bulls 7368 and 7369 contracted rinderpest, their temperatures rising on Oct. 17th, vesicles on Oct. 19th (7368) and 21st (7369). 7374 and 7377 failed to become infected but were susceptible. Infection present 6 and 18 hours after removal of the sick animals but not after 30 hours.

(b) Observations at Bareilly.

Tests similar to those described above were repeated at Bareilly. Two enclosures, one fully exposed and the other well shaded, were employed as before.

Throughout the experiments the weather was clear and bright with a maximum shade temperature varying from 100°F. when the observations were commenced, to about 75°F. at their close; the daily minimum temperature varied from 70° to 45°F. during the observations.

(1) *The duration of rinderpest infection in the enclosure without shade.*

Experiment 1.

1916, Oct. 15th	..	..	Hill bulls 7 and 8, third day of rinderpest attack, placed in the enclosure.
,, 19th, 10 A.M.	..	..	Sick bulls removed.
,, ,, 6 P.M.	(8 hours)	..	Hill bull 27 placed in the enclosure.
,, 20th, 6 A.M.	(20 ,,)	..	,, 28 ,, ,,
,, ,, 6 P.M.	(32 ,,)	..	,, 29 ,, ,,
,, 23rd	..	..	Test animals removed.

Result. The test animals failed to contract rinderpest; all were susceptible. Infection absent after 8 hours.

Experiment 2.

1916, Oct. 30th, 9 A.M.	..	..	Hill bulls 36 and 37, sixth day of rinderpest attack, placed in the enclosure.
,, 31st, 12 noon	..	..	Sick bulls removed.
,, ,, 6 P.M.	(6 hours)	..	Hill bull 49 placed in the enclosure.
,, ,, 12 midnight	(12 hours)		,, 52 ,, ,, ,,
Nov. 1st, 12 noon	(24 hours)	..	,, 48 ,, ,, ,,
,, ,, ,,	(,, ,,)	..	,, 49 removed.
,, 8th	..	..	Hill bulls 52 and 48 removed.

Result. Hill bull 49 contracted rinderpest. Hill bulls 52 and 48 failed to become infected; both were susceptible. Infection present 6 hours after removal of the sick animals but not after 12 hours.

Experiment 3.

1916, Nov. 8th	..	..	Hill bulls 43 and 44, sixth day of rinderpest attack, placed in the enclosure.
„ 10th, 10 A.M.	..	..	Sick bulls removed.
„ „ 2 P.M.	(4 hours)	..	Hill bull 63 placed in the enclosure.
„ „ 6 P.M.	(8 „)	..	„ 64 „ „ „
„ 11th, 6 A.M.	(20 „)	..	„ 29 „ „ „
„ „ „	..	..	„ 63 removed.
„ 20th	..	..	Hill bulls 64 and 29 removed.

Result. Hill bull 63 contracted rinderpest. Hill bulls 64 and 29 failed to become infected; 64 proved to be susceptible but 29 died on Nov. 22nd from pneumonia before its susceptibility could be tested.

Infection present 4 hours after removal of the sick animals but not after 8 hours.

(2) *The duration of rinderpest infection in the enclosure shaded by trees.*

Experiment 1.

1916, Oct. 14th	..	..	Hill bulls 5 and 6, fourth day of rinderpest attack, placed in the enclosure.
„ 17th, 10 A.M.	..	..	Sick bulls removed.
„ „ 6 P.M.	(8 hours)	..	Hill bull 21 placed in the enclosure.
„ 18th, 6 A.M.	(20 „)	..	„ 22 „ „ „
„ „ 6 P.M.	(32 „)	..	„ 26 „ „ „
„ 20th	..	..	„ 21 removed.
„ 26th	..	..	Hill bulls 22 and 26 removed.

Result. The test animals failed to contract rinderpest; 21 and 22 were susceptible; 26 died, with lesions of gastro-enteritis, before its susceptibility could be tested. Infection absent 8 hours after removal of the sick animals.

Experiment 2.

1916, Oct. 24th	..	..	Hill bulls 23 and 24, sixth day of rinderpest attack, placed in the enclosure.
„ 26th, 7 A.M.	..	..	24 died at this time. Its carcase and 23 were removed immediately.
„ „ 3 P.M.	(8 hours)	..	Hill bull 19 placed in the enclosure.
„ 27th, 3 A.M.	(20 „)	..	„ 14 „ „ „
„ „ 3 P.M.	(32 „)	..	„ 13 „ „ „
Nov. 1st	..	..	Test animals removed.

Result. Hill bull 14 (20 hours) contracted rinderpest, its temperature rising on the 5th day. Hill bulls 19 (8 hours) and 13 (32 hours) failed to become infected although later they proved to be susceptible; the escape of 19 must have been accidental. Infection present 20 hours after removal of the sick animals but not after an interval of 32 hours.

Experiment 3.

1916, Nov. 24th	..	..	Hill bulls 46 and 71, fifth day of rinderpest attack, placed in the enclosure.
,, 26th,	9 A.M.	..	.. Sick bulls removed.
,, ,,	5 P.M.	(8 hours)	.. Hill bull 76 placed in the enclosure.
,, 27th,	9 A.M.	(24 ,,)	.. ,, 81 ,, ,, ,,
,, 28th,	9 A.M.	(48 ,,)	.. ,, 77 ,, ,, ,,
,, 29th	..	..	Hill bulls 76 and 81 removed from the enclosure.
Dec. 8th	..	..	Hill bull 77 removed from the enclosure.

Result. Hill bulls 76 and 81 contracted rinderpest, their temperatures rising four and five days after exposure respectively; 77 failed to become infected but was susceptible. Infection present 8 and 24 hours after removal of the sick animals but not after an interval of 48 hours.

Summary.

Number of Experiment	Intervals after removal of the sick animals at which infectiveness was tested	Result
	1. *Ground exposed to direct sunlight.*	
	(a) *At Muktesar.*	
1	1 and 2 days ..	Infection absent.
2	16 and 20 hours ..	,, ,,
3	16, 20, and 24 hours ..	,, ,,
4	12, 24, and 30 hours ..	,, ,,
5	5, 8, and 20 hours ..	,, ,,
6	5, 8, and 20 hours ..	Infection present after 5 and 8 hours but not after 20 hours.
7	5, 8, and 20 hours ..	Infection absent.
	(b) *At Bareilly.*	
1	8, 20, and 32 hours ..	Infection absent.
2	6, 12, and 24 hours ..	Infection present after 6 hours but not after 12 and 24 hours.
3	4, 8, and 20 hours ..	Infection present after 4 hours but not after 8 and 20 hours.
	2. *Ground shaded by trees.*	
	(a) *At Muktesar.*	
1	2, 3, and 4 days ..	Infection absent.
2	44, 56, and 72 hours ..	,, ,,
3	32, 48, and 56 hours ..	,, ,,
4	6, 18, 30 and 48 hours ..	Infection present after 6 hours but not after 18, 30, and 48 hours.
5	6, 18, 30, and 48 hours ..	Infection present after 6 and 18 hours but not after 30 and 48 hours.
	(b) *At Bareilly.*	
1	8, 20, and 32 hours ..	Infection absent.
2	8, 20, and 32 hours ..	Infection present after 20 hours but not after 32 hours.
3	8, 24, and 48 hours ..	Infection present after 24 hours but not after 48 hours.

Conclusions.

(1) In the hills open ground free from vegetation and exposed to direct sunlight, when heavily infected by animals suffering from rinderpest, was found to be infective for healthy cattle eight hours after removal of the sick animals but not for a longer period. In the plains under similar conditions infection did not persist for more than six hours.

(2) When protection from direct sunlight was provided the rinderpest virus was found to survive on the ground in the hills for 18 hours and in the plains for 24 hours but not for longer periods. In some of the experiments infection was absent after similar or shorter intervals of time.

III. DURATION OF RINDERPEST INFECTION IN CLOSED SHEDS.

(a) Observations at Muktesar.

In order that the conditions under which the tests were carried out might be as variable as possible, two sheds were utilized. One was a well-built masonry stable with slate and cement floor (Plate III) and the other a rough wattle-and-mud erection with thatched roof and plain earth floor (Plate IV), the latter being the common type of cattle shed found in the hill districts.

Hill bulls suffering from rinderpest were placed in the sheds to infect them and were left there until a short time before death ; no litter was taken from the sheds during an observation. At intervals after the removal of the sick animals healthy hill bulls were turned into the sheds and left for at least 24 hours to test whether the rinderpest virus was still capable of causing infection. Later the test animals were isolated in company with healthy bulls to control the possibility of outside infection. The susceptibility of all animals failing to react was subsequently tested by an injection of virulent blood or successful exposure to natural infection in later experiments.

Post-mortem examinations and, if necessary, blood inoculations, were carried out in the case of all animals which died without having shown diagnostic symptoms during life.

During the observations the daily maximum shade temperature varied from 56° to 73°F. and the minimum from 37° to 57°F.

(1) *Tests in stone stable.*

Experiment 1.

1916, July 28th	..	..	Hill bulls 6948 and 6949, fourth day of rinderpest attack, placed in the stable.
,, 31st, 10-45 A.M.	..	..	Sick bulls removed.
Aug. 4th, 10-45 A.M.	(4 days)	..	Hill bull 7014 placed in the stable.
,, 5th, 10-45 A.M.	(5 ,,)	..	,, ,, 7020 ,, ,, ,,
,, 6th, 10-45 A.M.	(6 ,,)	..	,, ,, 7023 ,, ,, ,,
,, ,, ,, ,,	(,, ,,)	..	,, ,, 7014 removed.
,, 7th	..	..	,, ,, 7020 ,,
,, 16th	..	..	,, ,, 7023 ,,

Result. The test animals failed to contract rinderpest ; all were susceptible. Infection absent after four days.

Experiment 2.

1916, Aug. 28th	..	..	Hill bulls 7013 and 7068, fourth day of rinderpest attack, placed in the stable.
Sept. 1st,	11 A.M. ..	..	Sick bulls removed.
,, 2nd,	7 P.M. (32 hours)	..	Hill bull 7033 placed in the stable.
,, 3rd,	7 P.M. (56 ,,)	..	,, ,, 7176 ,, ,, ,,
,, 4th,	7 P.M. (80 ,,)	..	,, ,, 7184 ,, ,, ,,
,, ,,	,, (,, ,,)	..	,, ,, 7033 removed.
,, 5th,	..	..	,, ,, 7176 ,,
,, 11th,	..	..	,, ,, 7184 ,,

Result. The test animals failed to contract rinderpest ; all were susceptible. Infection absent after 24 hours.

Experiment 3.

1916, Oct. 22nd	..	..	Hill bulls 7391 and 7392, fourth day of rinderpest attack, placed in the stable.
,, 24th, 11 A.M.	..	..	Sick bulls removed.
,, 25th, 11 A.M.	(1 day)	..	Hill bull 7432 placed in the stable.
,, 26th, 11 A.M.	(2 days)	..	,, ,, 7446 ,, ,, ,,
,, 27th, 11 A.M.	(3 ,,)	..	,, ,, 7450 ,, ,, ,,
,, ,, ,,	(,, ,,)	..	,, ,, 7432 removed from the stable.
,, 31st	..	..	Hill bulls 7446 and 7450 removed.

Result. The test animals failed to contract rinderpest ; all were susceptible. Infection absent after 24 hours.

Experiment 4.

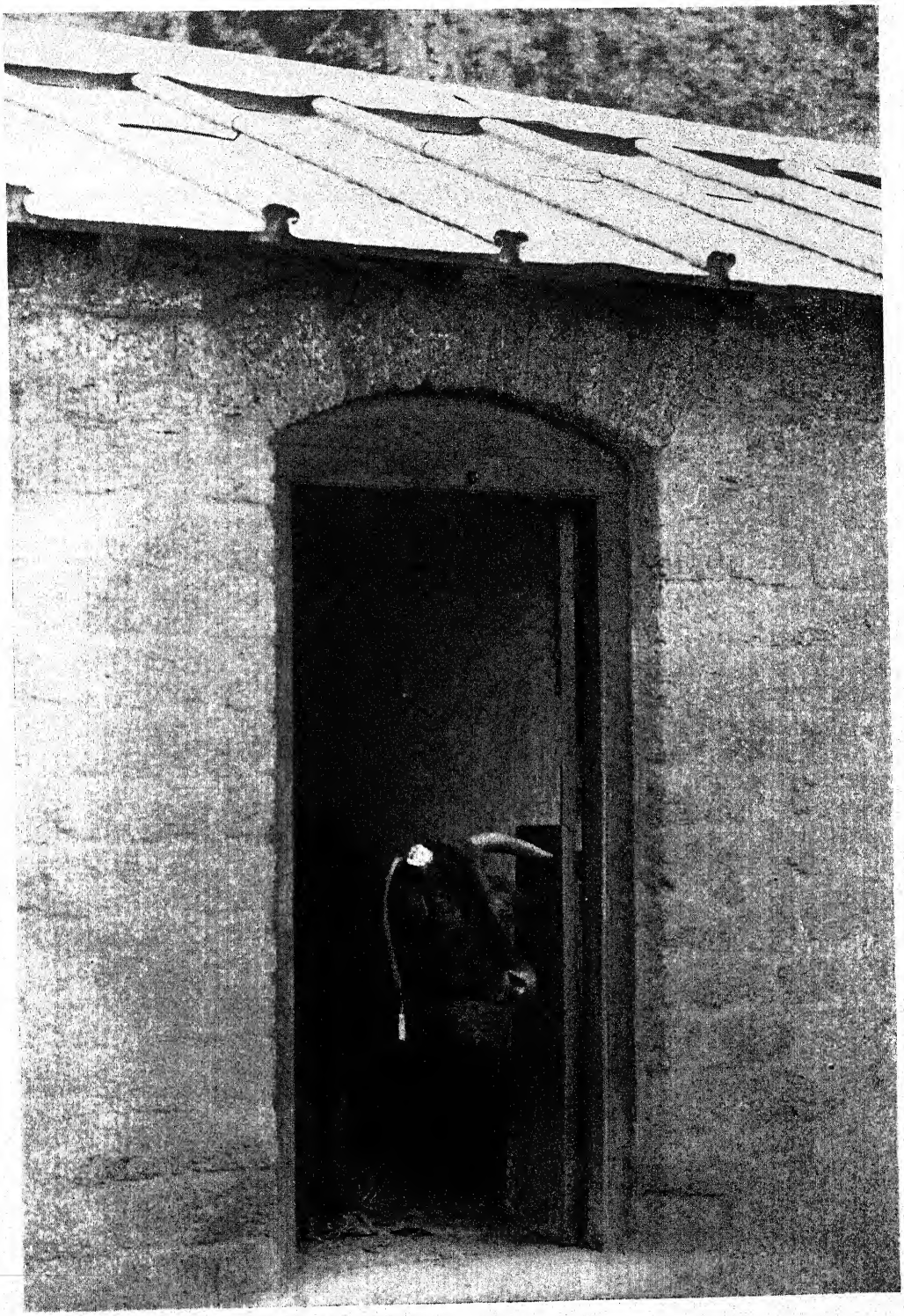
1916, Dec. 2nd	..	..	Hill bulls 7593 and 7594, fourth day of rinderpest attack, placed in the stable.
,, 5th, 12 noon	..	..	Sick bulls removed.
,, ,, 6 P.M.	(6 hours)	..	Hill bull 7627 placed in the stable.
,, 6th, 6 A.M.	(18 ,,)	..	,, ,, 7628 ,, ,, ,,
,, ,, 6 P.M.	(30 ,,)	..	,, ,, 7629 ,, ,, ,,
,, 10th	..	..	Hill bulls 7627 and 7628 removed from the stable.
,, 16th	..	..	Hill bull 7629 removed from the stable.

Result. Hill bulls 7627 and 7628 contracted rinderpest ; 7629 failed to become infected but was susceptible. Infection present 6 and 18 hours after removal of the sick animals but not after 30 hours.

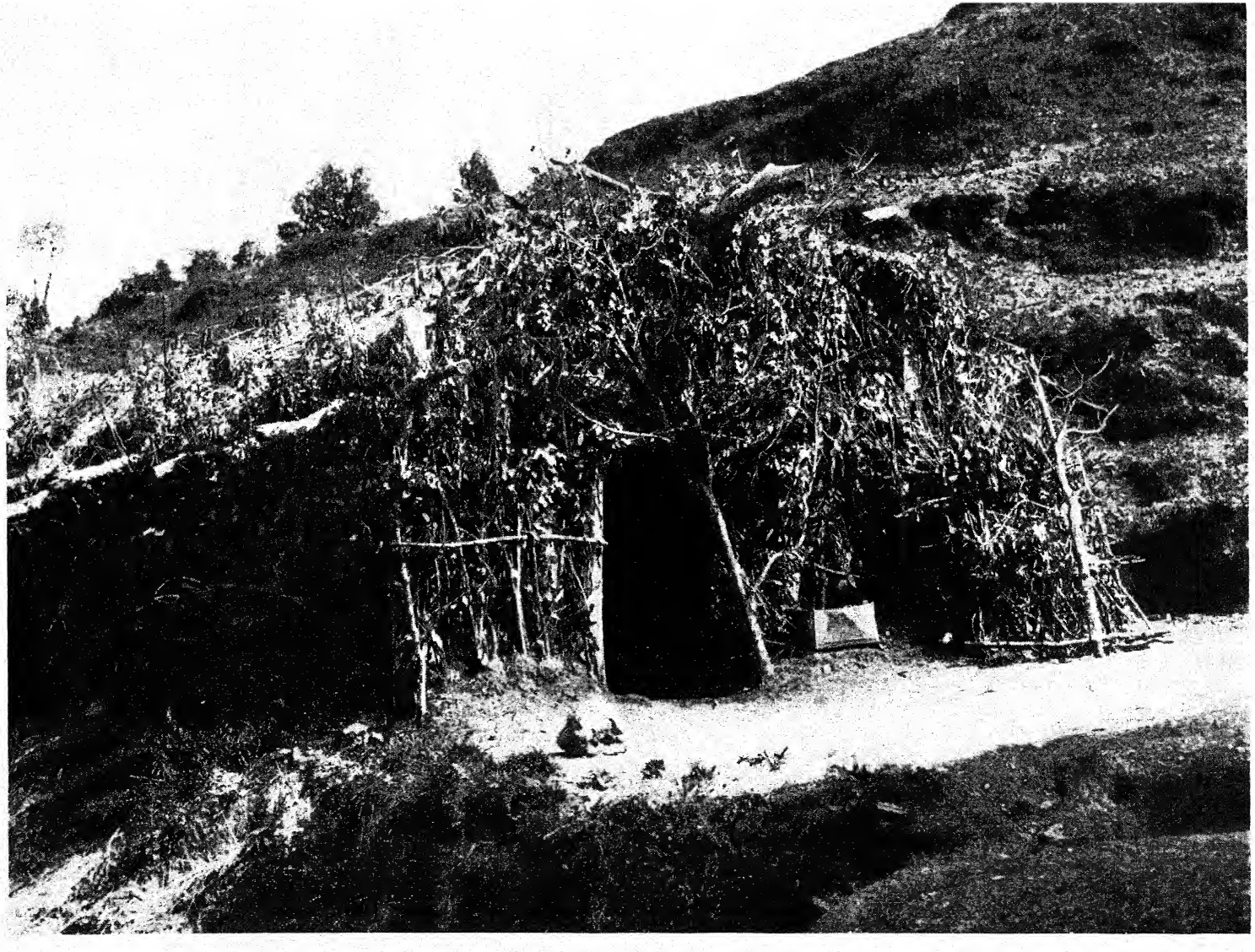
(2) *Tests in the wattle-and-mud shed.*

Experiment 1.

1916, April 17th	..	..	Hill bulls 6742 and 6743 inoculated with virulent rinderpest blood and placed in the shed.
,, 22nd	..	..	Temperature of both bulls over 40°C.
,, 23rd to 26th	..	..	Bulls showing vesicles in mouth and diarrhœa.



THE STONE STABLE USED IN THE TESTS AT MUKTESAR.



THE WATTLE-AND-MUD SHED USED IN THE TESTS AT MUKTESAR.

1916, April 27th	..	..	Both animals died early in the morning. Carcases removed at 10 A.M.
,, 29th, 10 A.M.	(2 days)	..	Hill bull 6762 placed in the shed.
May 1st, 10 A.M.	(4 ,,)	..	,, ,, 6769 ,, ,, ,,
,, 3rd	..	..	,, ,, 6762 removed.
,, ,, 10 A.M.	(6 days)	..	,, ,, 6774 placed in the shed.
,, ,, ,,	,, ,,	..	,, ,, 6769 removed.
,, 12th	..	..	,, ,, 6774 removed.

Result. Hill bull 6762 contracted rinderpest (May 4th temperature 40°C., May 7th vesicles). 6769 and 6774 failed to become infected. 6774 proved to be susceptible, but 6769 died four days after being inoculated with virulent blood and a *post-mortem* examination failed to show any lesions of rinderpest. Infection present two days after removal of the dead animals, but absent after four days.

Experiment 2.

1916, July 1st	..	..	Hill bulls 6872 and 6873, seventh day of the disease, vesicles and diarrhœa, placed in the shed.
,, 3rd, 11 A.M.	..	..	Sick bulls removed.
,, 6th, 11 A.M.	(3 days)	..	Hill bull 6918 placed in the shed.
,, 7th, 11 A.M.	(4 ,,)	..	,, ,, 6921 ,, ,, ,,
,, 8th, 11 A.M.	(5 ,,)	..	,, ,, 6925 ,, ,, ,,
,, ,, ,,	,, ,,	..	,, ,, 6918 removed.
,, 10th	..	..	Hill bulls 6921 and 6925 removed.

Result. The test animals failed to contract rinderpest ; all were susceptible. Infection absent after three days.

Experiment 3.

1916, Oct. 1st	..	..	Hill bulls 7298 and 7300, fifth day of rinderpest attack, vesicles and diarrhœa, placed in the shed.
,, 4th, 11 A.M.		..	Sick bulls removed.
,, 5th, 11 A.M.	(24 hours)	..	Hill bull 7340 placed in the shed.
,, 6th, 11 A.M.	(48 ,,)	..	,, ,, 7344 ,, ,, ,,
,, ,, ,,	,, ,,	..	,, ,, 7340 removed.
,, ,, 8 P.M.	(57 hours)	..	,, ,, 7345 placed in the shed.
,, 13th, 8 A.M.	..	..	,, ,, 7344 removed.
,, 17th	..	..	,, ,, 7345 ,,

Result. Hill bulls 7340 and 7344 contracted rinderpest. 7345 failed to become infected but was susceptible. Infection present 24 and 48 hours after removal of the sick animals but not after 57 hours.

(b) Observations at Bareilly.

For these tests two stables (Nos. 1 and 2) of similar construction were employed. Each was built of brick, closed on three sides but open on the

fourth, with tiled roof and earth floor. The tests were carried out as in the previous experiments. The variations in shade temperatures were the same as noted under the tests of ground infection.

EXPERIMENT 1.

1916, Oct. 6th	..	..	Hill bulls 1 and 2 inoculated subcutaneously with 1 c.c. virulent rinderpest blood and placed in stable No. 1.
„ 12th	..	..	Bulls showing vesicles in the mouth, temperatures over 40°C. and suffering from diarrhœa.
„ 14th, 6 A.M.		..	Sick bulls removed from the stable.
„ „ 6 P.M.	(12 hours)	..	Hill bull 13 placed in the stable.
„ 15th, 6 A.M.	(24 hours)	..	„ „ 14 „ „ „
„ 16th, 6 A.M.	(48 hours)	..	„ „ 19 „ „ „
„ 22nd	..	..	Test animals removed from the stable.

Result. The test animals failed to contract rinderpest; all were susceptible. Infection absent 12 hours after removal of the sick animals.

EXPERIMENT 2.

1916, Oct. 6th	..	..	Hill bulls 3 and 4 inoculated subcutaneously with 1 c.c. virulent rinderpest blood and placed in stable No. 2.
„ 12th	..	..	Sick bulls showing vesicles in mouth and diarrhœa.
„ 14th, 6 A.M.	..	..	Hill bull 3 died at 5 A.M. Its carcase and sick bull 4 were removed at 6 A.M.
„ „ 6 P.M.	(12 hours)	..	Hill bull 12 placed in the stable.
„ 15th, 6 A.M.	(24 hours)	..	„ „ 15 „ „ „
„ 16th, 6 A.M.	(48 hours)	..	„ „ 18 „ „ „
„ 18th	..	..	Hill bulls 15 and 18 removed from the stable. 12 left in the stable to reinfect it, see Experiment 3.

Result. Hill bull 12 contracted rinderpest, its temperature rising on Oct. 18th. Hill bulls 15 and 18 failed to become infected though both were susceptible. Infection present 12 hours after removal of the sick animals but absent after 24 hours.

EXPERIMENT 3.

1916, Oct. 18th	..	.. Hill bulls 10 and 12, fourth day of rinderpest attack, placed in stable No. 2.
„ 19th	..	.. Hill bull 16, fourth day of rinderpest attack, placed in stable No. 2.
„ 20th	..	.. Sick bulls showing vesicles in mouth and diarrhœa.
„ 21st, 6 A.M.	..	.. Sick bulls removed from the stable.

1916, Oct. 21st, 6 P.M.	(12 hours)	..	Hill bull 30 placed in the stable.
,, 22nd, 6 A.M.	(24 hours)	..	,, ,, 31 ,, ,, ,,
,, 23rd, 6 A.M.	(48 hours)	..	,, ,, 33 ,, ,, ,,
,, 28th	..	..	,, ,, 30 removed from the stable.
Nov. 4th	..	..	Hill bulls 31 and 33 removed from the stable.

Result. Hill bull 30 contracted rinderpest, its temperature rising on Oct. 28th. Hill bulls 31 and 33 failed to become infected; both were susceptible. Infection present 12 hours after removal of the sick animals but absent after 24 hours.

Experiment 4.

1916, Oct. 22nd	..	..	Hill bulls 6 and 25, sixth and fourth days of rinderpest attack, placed in stable No. 1.
,, 24th, 10 A.M.	..	..	Sick bulls removed from the stable.
,, ,, 6 P.M.	(8 hours)	..	Hill bull 38 placed in the stable.
,, 25th, 6 A.M.	(20 hours)	..	,, ,, 39 ,, ,, ,,
,, 26th, 6 A.M.	(44 hours)	..	,, ,, 40 ,, ,, ,,
,, 30th	..	..	Test animals removed from the stable.

Result. Hill bull 38 contracted rinderpest, its temperature rising on Oct. 30th. Hill bulls 39 and 40 failed to become infected; both were susceptible. Infection present 8 hours after removal of the sick animals but absent after 20 hours.

Experiment 5.

1916, Nov. 6th	..	..	Hill bulls 13 and 42, fifth day of rinderpest attack, placed in stable No. 2.
,, 7th	..	..	Sick bulls showing vesicles in mouth and diarrhœa.
,, 8th, 10 A.M.	..	..	Sick bulls removed from the stable.
,, ,, 6 P.M.	(8 hours)	..	Hill bull 58 placed in the stable.
,, 9th, 6 A.M.	(20 hours)	..	,, ,, 28 ,, ,, ,,
,, ,, 6 P.M.	(32 hours)	..	,, ,, 48 ,, ,, ,,
,, 12th	..	..	Test animals removed from the stable.

Result. Hill bulls 58 and 28 contracted rinderpest, their temperatures rising on Nov. 13th and 14th respectively. Hill bull 48 failed to become infected but was susceptible.

Infection present 8 and 20 hours after the removal of the sick animals but not after 32 hours.

Experiment 6.

1916, Nov. 16th, 9 A.M.	..	..	Hill bulls 47 and 51, both naturally infected and showing vesicles in the mouth and diarrhœa, were placed in stable No. 1.
,, 17th, 10 A.M.	..	..	Sick bulls removed from the stable.

1916, Nov.	17th, 6 P.M.	(8 hours)	..	Hill bull 68 placed in the stable.
„	18th, 6 A.M.	(20 hours)	..	„ „ 46 „ „ „
„	„ 6 P.M.	(32 hours)	..	„ „ 57 „ „ „
„	19th, 6 P.M.	..	..	„ „ 68 removed from the stable.
„	22nd	..	..	„ „ 46 „ „ „ „
Dec.	2nd	..	..	„ „ 57 „ „ „ „

Result. Hill bulls 68 and 46 contracted rinderpest, their temperatures rising four days after exposure to the infection. Hill bull 57 failed to become infected but was susceptible. Infection present 8 and 20 hours after removal of the sick animals but not after 32 hours.

SUMMARY.

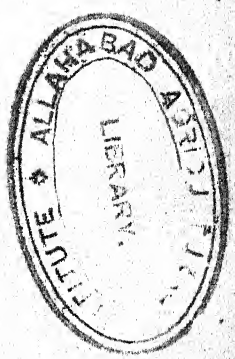
Number of Experiment	Intervals after removal of sick animals at which infectiveness was tested	Result
	(a) *In stone stable at Muktesar.*	
1	4, 5, and 6 days ..	Infection not present.
2	32, 56, and 80 hours ..	Do.
3	24, 48, and 72 hours ..	Do.
4	6, 18, and 30 hours ..	Infection present after 6 and 18 hours but absent after 30 hours.
	(b) *In wattle-and-mud shed at Muktesar.*	
1	2, 4, and 6 days ..	Infection present after two days but absent after four or six days.
2	3, 4, and 5 days ..	Infection not present.
3	24, 48, and 57 hours ..	Infection present after 24 and 48 hours but absent after 57 hours.
	(c) *In open stables at Bareilly.*	
1	12, 24, and 48 hours ..	Infection not present.
2	12, 24, and 48 hours ..	Infection present after 12 hours but absent after 24 and 48 hours.
3	12, 24, and 48 hours ..	Infection present after 12 hours but absent after 24 and 48 hours.
4	8, 20, and 44 hours ..	Infection present after 8 hours but absent after 20 and 44 hours.
5	8, 20, and 32 hours ..	Infection present after 8 and 20 hours but absent after 32 hours.
6	8, 20, and 32 hours ..	Infection present after 8 and 20 hours but absent after 32 hours.

Conclusions.

(1) In the hills, rinderpest infection was found to persist in a stone stable with slate floor for 18 hours after removal of the sick animals, but not for longer periods.

(2) In a wattle-and-mud shed with earth floor and no windows, in the same situation, rinderpest infection persisted for 48 hours after removal of the sick animals, but not for longer periods.

(3) In the plains, open stables were found to be infective up to 20 hours after removal of the sick animals, but not for longer periods.



IV. DURATION OF VITALITY OF THE RINDERPEST VIRUS IN FÆCES, URINE AND MUCUS DISCHARGES.

In the foregoing observations the method of testing the presence of rinderpest infection by placing healthy animals on the infected areas, although simulating natural conditions most closely, does not allow of precise conclusions being drawn as to the vitality of the virus owing to the possibilities of accidental avoidance of the infected material by the test animals. The following experiments were therefore carried out to test more accurately the length of time that the virus can maintain its vitality in fæces, urine and mucus discharges from animals suffering from rinderpest.

(a) Observations made at Muktesar.

The material was collected from sick animals at the height of an attack and kept at room temperature in open vessels. At intervals portions of it mixed with a small quantity of meal were fed to healthy cattle. Owing to failure to produce infection in the first two experiments by this method of administration some of the material was also rubbed into the nostrils and on the muffle of the test animals in the subsequent observations. The test animals were then isolated with healthy controls. The daily maximum shade temperature varied from 67° to 73° F. and the minimum from 54° to 57° F. during the observations.

Experiment 1.

1916, July 26th, 11-30 A.M.	..	..	Urine (12 oz.) collected from hill bull 6963, sixth day of rinderpest attack, vesicles in mouth and diarrhœa, temperature 40·3 C.
,, ,, 7 P.M.	..	..	Fæces (10 oz.) collected from hill bull 6963, temperature 39·8° C.
,, 28th, 11-30 A.M.	(48 hours)	..	Urine (6 oz.) fed to hill bull 6987.
,, ,, 7 P.M.	(48 hours)	..	Fæces (5 oz.) ,, ,, ,, 6988.
,, 29th, 11-30 A.M.	(72 hours)	..	Urine (6 oz.) ,, ,, ,, 6989.
,, ,, 7 P.M.	(72 hours)	..	Fæces (5 oz.) ,, ,, ,, 6990.

Result. None of the test animals became infected ; all were susceptible. Urine and fæces from sick bull 6963 after 48 hours' storage proved non-infective when fed to healthy cattle.

Experiment 2.

1916, Aug. 12th,	8-30 A.M.	..	.. Fæces (6 oz.) collected from hill bull 7024, sixth day of rinderpest attack, vesicles in mouth and diarrhœa, temperature 39·3° C.
,, ,,	11 A.M.	..	.. Urine (4 oz.) collected.
,, 15th,	11 A.M.	(24 hours)	.. Urine (2 oz.) fed to hill bull 7012.
,, ,,	8 P.M.	(33 hours)	.. Urine (2 oz.) ,, ,, ,, 7011.
,, ,,	8-30 P.M.	(36 hours)	.. Fæces (3 oz.) ,, ,, ,, 7013.
,, 14th,	8-30 P.M.	(48 hours)	.. ,, (3 oz.) ,, ,, ,, 7068.

Result. None of the test animals became infected; all were susceptible. Urine from sick bull 7024 was not infective 24 hours after collection. Fæces from the same animal was not infective 36 hours after collection. The tests in both cases were made by mixing the material with the animal's food.

Experiment 3.

1916, Aug. 23rd,	11-30 A.M.	..	.. Fæces, saliva and nasal discharge collected from hill bull 7071, seventh day of rinderpest attack, vesicles in mouth and diarrhœa, temperature 39·4°C.
,, 24th,	3-30 P.M.	(28 hours)	.. Saliva and nasal discharge given to hill bull 7013.
,, ,,	,,	,,	.. Fæces given to hill bull 7068.
,, 25th,	7-30 A.M.	(44 hours)	.. Saliva and nasal discharge given to hill bull 7012.
,, ,,	,,	,,	.. Fæces given to hill bull 7011.

Result. All the test animals contracted rinderpest, their temperatures rising on the third or fourth day. Mucous discharge and fæces from sick bull 7071 proved to be infective 28 and 44 hours after collection. In this, and the subsequent observations, the material was rubbed into the nostrils of the test animals in addition to being mixed with their food.

Experiment 4.

1916, Aug. 30th,	12 noon	..	.. Fæces, saliva and nasal discharge collected from hill bull 7087, seventh day of rinderpest attack, vesicles in mouth and diarrhœa, temperature 39·0°C.
,, 31st,	5 P.M.	(29 hours)	.. Saliva and nasal discharge given to hill bull 7020.
,, ,,	,,	,,	.. Fæces given to hill bull 7014.
Sept. 1st,	12 noon	(48 hours)	.. Saliva and nasal discharge given to hill bull 7081.
,, ,,	6 P.M.	(54 hours)	.. Saliva and nasal discharge given to hill bull 7165.
,, ,,	,,	,,	.. Fæces given to hill bull 7164.

Result. Hill bulls 7020, 7014, 7023, and 7164 contracted rinderpest. 7081 and 7165 failed to become infected but were susceptible. Saliva from

sick bull 7087 was infective 29 hours after collection but had lost its infectiveness 48 hours after collection. Fæces from the same animal proved to be infective 29, 48, and 54 hours after collection.

EXPERIMENT 5.

1916, Sept.	1st, 12 noon	..	.. Fæces, saliva and nasal discharge collected from hill bulls 7104 and 7108, seventh day of rinderpest attack, vesicles in mouth and diarrhœa, temperatures 39·8°C. and 37·8°C. The discharges from both animals were mixed.
,,	3rd, 12 noon	(48 hours)	.. Mixed fæces and mucous discharges given to hill bull 7175.
,,	4th, 6 A.M.	(66 hours)	.. Mixed fæces and mucous discharges given to hill bull 7177.
,,	,, 6 P.M.	(78 hours)	.. Mixed fæces and mucous discharges given to hill bull 7183.

Result. None of the test animals became infected; all were susceptible. Mixed fæces, saliva and nasal discharge from sick bulls 7104 and 7108 were not infective 48 hours after collection.

EXPERIMENT 6.

1916, Sept.	10th, 11 A.M.	..	Fæces collected from hill bull 7182, sixth day of rinderpest attack, vesicles in mouth and diarrhœa, temperature 38·9°C.
,,	11th, 5 P.M.	(20 hours)	.. Fæces given to hill bull 7174.
,,	12th, 11 A.M.	(48 hours)	.. ,, ,, ,, ,, 7173.
,,	13th, 11 A.M.	(72 hours)	.. ,, ,, ,, ,, 7026.

Result. Hill bulls 7174 and 7173 contracted rinderpest, temperatures rising on the fifth and sixth days respectively. Hill bull 7026 did not become infected, but was susceptible. Fæces from sick bull 7182 were infective 20 and 48 hours after collection but not after 72 hours.

(b) OBSERVATIONS MADE AT BAREILLY.

As the purpose of the tests with discharges from sick animals was to control the observations carried out in the enclosures and various buildings under more natural conditions, the procedure followed at Bareilly was to collect and mix the material and divide it into two portions; one-half was then stored in the shade at air temperature and the other exposed to direct sunlight in the open. At intervals the infectiveness of each portion was tested on healthy hill bulls as in the experiments at Muktesar.

The daily maximum shade temperature varied from 82° to 95° F. and the minimum from 58° to 68° F., during the observations.

Experiment 1.

1916, Oct. 31st, 6-30 A.M.	..	..	Fæces and urine collected from hill bulls 34 and 37, seventh day of rinderpest attack, both showing vesicles in the mouth and diarrhœa. Materials mixed. One portion of the mixture kept at room temperature and the other exposed to direct sunlight; depth of the fluid about one and a half inches.	
,, ,, 12-30 P.M. (6 hours)	..	Mixture exposed to sun given to hill bull		27.
,, ,, 6-30 P.M. (12 hours)	..	,, ,,	,, ,, ,,	28.
,, ,, ,, ,,	..	,, in shade	,, ,, ,,	51.
Nov. 1st, 6-30 A.M. (24 hours)	..	,, ,,	,, ,, ,,	53.
,, ,, 6-30 P.M. (36 hours)	..	,, exposed to sun	,, ,, ,,	29.
,, 2nd, 6-30 A.M. (48 hours)	..	,, in shade	,, ,, ,,	46.

Result. Hill bull 27 contracted rinderpest, its temperature rising on Nov. 6th. None of the other test animals became infected though all were susceptible. Mixed fæces and urine from sick bulls 34 and 37 exposed to the sun remained infective for six hours but not for 12 or 36 hours. In the shade the same material was non-infective after 12 hours.

Experiment 2.

1916, Nov. 18th, 6-30 A.M.	..	..	Fæces and urine collected from hill bulls 28 and 70, ninth day of rinderpest attack, both showing vesicles in mouth and diarrhœa. Fæces and urine mixed. Part kept in shade and part exposed to sunlight.	
,, ,, 12-30 A.M. (6 hours)	..	Mixture exposed to sun given to hill bull		61.
,, ,, ,, ,,	..	,, in shade	,, ,, ,,	65.
,, ,, 6-30 P.M. (12 hours)	..	,, exposed to sun	,, ,, ,,	62.
,, ,, ,, ,,	..	,, in shade	,, ,, ,,	71.
,, 19th, 6-30 P.M. (36 hours)	..	,, exposed to sun	,, ,, ,,	72.
,, ,, ,, ,,	..	,, in shade	,, ,, ,,	73.

Result. Hill bulls 61, 65, 71, and 73 contracted rinderpest, their temperatures rising on the third or fourth day after receiving the material. Hill bulls 62 and 72 failed to become infected but were susceptible.

Mixed fæces and urine from sick bulls 28 and 70 kept in the shade were infective 6, 12, and 36 hours after collection. The same material exposed to direct sunlight was infective 6 hours after collection but was non-infective after 12 and 36 hours' exposure.

Experiment 3.

1916, Nov. 25th,	10 A.M.	..	..	Fæces and urine collected from hill bulls 46, 61, and 71, seventh day of rinderpest attack, all showing vesicles in mouth and diarrhœa. Fæces and urine mixed. Part exposed to sunlight and part kept in shade as in previous experiments.
,, ,,	6 P.M.	(8 hours)	..	Mixture exposed to sun given to hill bull 83.
,, ,,	,,	,,	..	,, in shade ,, ,, ,, 85.
,, 26th,	10 A.M.	(24 hours)	..	,, exposed to sun ,, ,, ,, 74.
,, ,,	,,	,,	..	,, in shade ,, ,, ,, 75.
,, 27th,	,,	(48 hours)	..	,, exposed to sun ,, ,, ,, 80.
,, ,,	,,	,,	..	,, in shade ,, ,, ,, 78.
,, 28th,	,,	(72 hours)	..	,, ,, ,, ,, ,, 79.

Result. Hill bulls 83, 85, and 75 contracted rinderpest; 74, 80, 78, and 79 failed to become infected but were susceptible.

Mixed fæces and urine from sick bulls 46, 61, and 71 kept in the shade were infective 8 and 24 hours after collection, but were non-infective after 48 and 72 hours.

The same material exposed to direct sunlight was infective 8 hours after collection but was non-infective after 24 and 48 hours' exposure.

Summary.

(a) *Observations at Muktesar.*

No. of Experiment	Material	Intervals after collection at which infectiveness was tested	Result
1	Fæces ..	48 and 72 hours ..	Not infective.
	Urine ..	,, ,, ..	
2	Fæces ..	36 and 48 hours ..	
	Urine ..	24 and 33 hours ..	
3	Fæces ..	28 and 44 hours ..	Infective after both intervals.
	Saliva and nasal discharge	,, ,, ..	,, ,, ,,
4	Fæces ..	29, 48, and 54 hours ..	Infective after all intervals.
	Saliva and nasal discharge	,, ,, ..	Infective after 29 hours, but not after 48 and 54 hours.
5	Fæces, saliva and nasal discharge mixed	48, 66 and 78 hours ..	Not infective.
	Fæces ..	30, 48, and 72 hours ..	Infective after 30 and 48 hours, but not after 72 hours.

(b) *Observations at Bareilly.*

No. of Experiment	Conditions under which mixed fæces and urine were kept	Intervals after collection at which infectiveness was tested	Result
1	In shade ..	12, 24, and 48 hours ..	Not infected.
	In sun ..	6, 12, and 36 hours ..	Infective after 6 hours, but not after 12 and 36 hours.
2	In shade ..	,, ,, ,, ..	Infective after all intervals.
	In sun ..	,, ,, ,, ..	Infective after 6 hours, but not after 12 or 36 hours.
3	In shade ..	8, 24, 48, and 72 hours ..	Infective after 8 and 24 hours, but not after 48 and 72 hours.
	In sun ..	8, 24, and 48 hours ..	Infective after 8 hours, but not after 24 and 48 hours.

Conclusions.

(1) At Muktesar fæces and urine collected from animals suffering from rinderpest during the later stages of the disease and stored in the shade at air temperatures varying from 54° to 73° F. remained infective to healthy animals up to 54 hours after collection, but frequently these materials become non-infective after shorter intervals. In the plains at slightly higher temperatures, fæces and urine were found to be non-infective beyond 36 hours.

These observations confirm the results obtained by exposure of healthy cattle in sheds, and in enclosures shaded by trees at intervals after the removal of sick animals.

(2) When exposed to direct sunlight the virus survived in mixed fæces and urine for 8 hours but not for 12 hours; in the same materials kept in the shade the virus survived for 36 hours. The results obtained in infected enclosures, without shade, are therefore confirmed.

(3) The rinderpest virus survived in mixed saliva and nasal discharge kept at room temperature for 44 hours. It appeared to be less resistant in these discharges than in fæces and urine, since in one observation the infectiveness of saliva and nasal discharge was lost 48 hours after collection although fæces and urine from the same animal remained infective for 54 hours.

V. THE LENGTH OF TIME AFTER THE DEATH OF THE ANIMAL THAT RINDERPEST VIRUS CAN SURVIVE IN MEAT, BLOOD, AND BONE MARROW UNDER NATURAL CONDITIONS.

In addition to the excreta and mucus discharges of sick animals, the carcases of those that have died from rinderpest have also to be taken into consideration as possible sources of infection to others, especially as such carcases are commonly left to be broken up and carried away by birds and beasts of prey; it is important, therefore, to know how long the rinderpest virus can survive in the animal tissues after death.

A large number of observations have recently been made on the preservation of the rinderpest virus in a virulent condition in connection with the immunization of Government cattle by the serum simultaneous method, but only those carried out with material stored under natural conditions will be recorded in the present report.

All the tests were carried out at the Muktesar Laboratory. Blood for these observations was usually taken from a hill bull in the last stages of an attack of rinderpest and about 500 c.c. kept in an open dish at room temperature without protection of any kind; its infectiveness was tested at intervals by injecting 1 c.c. subcutaneously into a healthy hill bull.

Meat and bones were removed as soon as an animal in the last stages of the disease had been bled to death, and both were left exposed at room temperature. The meat was cut from the hind quarters in pieces of about 6 to 8 lb. weight; the survival of the rinderpest virus was tested by cutting out a small piece from the depth of the tissue, crushing it in a mortar with a few cubic centimetres of normal saline solution and injecting the fluid into a healthy hill bull.

The infectiveness of the bones was tested by sawing one of these open, removing the marrow and mixing it with normal saline solution; the fluid, filtered through muslin, was then injected subcutaneously into a healthy hill bull.

Experiment 1.

1916, Sept. 1st, 12-30 P.M. ..	.. Hill bull 7108, seventh day of rinderpest attack, showing vesicles in the mouth, diarrhœa, and in a dying condition bled to death. Blood and meat from hind quarters kept in open dishes at room temperature.
,, 4th, 12-30 P.M. (3 days)	.. Hill bull 7181 inoculated subcutaneously with 1 c.c. blood of 7108.
,, ,, ,, ,,	.. Hill bull 7182 inoculated subcutaneously with 5 c.c. extract from meat of 7108.
,, 7th, 12-30 P.M. (6 days)	.. Hill bull 7193 inoculated subcutaneously with 1 c.c. blood of 7108.
,, ,, ,, ,,	.. Hill bull 7104 inoculated subcutaneously with 5 c.c. extract from meat of 7108.
,, 11th, 12-30 P.M. (10 days)	.. Hill bull 7183 inoculated subcutaneously with 5 c.c. extract from meat of 7108.

The air temperature throughout this experiment varied from 54° to 73°F. Both the blood and meat became putrid very quickly ; putrefactive organisms were very numerous in the blood after three days' storage and in the meat after six days' storage.

Result. Hill bull 7182 contracted rinderpest, its temperature rising on the third day after inoculation. The other test animals failed to become infected though all were susceptible. Blood from hill bull 7108 stored at room temperature without protection had lost its infectiveness three days after collection.

Meat from hill bull 7108 kept exposed at room temperature was infective after three days but had lost its infectiveness six days after the death of the animal.

Experiment 2.

1916, Sept. 14th, 11-45 A.M. ..	.. Hill bull 7192, seventh day of rinderpest attack, vesicles in mouth and diarrhœa, bled to death. Blood, meat and bones stored at room temperature in open dishes.
,, 16th (2 days) ..	.. Hill bull 7193 injected with 1 c.c. blood.
,, 17th (5 days) ..	.. ,, ,, 7252 ,, ,, 1 c.c. ,,
	,, ,, 7253 ,, ,, 3 c.c. extract of the meat.
,, 18th (4 days) ..	.. Hill bull 7194 ,, ,, 1 c.c. blood.
,, 19th (5 days) ..	.. ,, ,, 7267 ,, ,, 3 c.c. extract of the bone marrow.
	Hill bull 7268 ,, ,, 6 c.c. extract of the meat.
,, 23rd (9 days) ..	.. Hill bull 7223 ,, ,, 4 c.c. extract of the bone marrow.
	Hill bull 7165 ,, ,, 7 c.c. extract of the meat.
	Hill bull 7033 ,, ,, 1 c.c. blood.

The air temperature varied from 52° to 65° F. Putrefaction of the materials set in very quickly; it was marked in the blood at 3 days and in the meat and bone marrow at 5 days.

Result. Hill bulls 7193, 7194, and 7033 injected with blood, 7267 and 7223 injected with bone marrow and 7253 and 7268 injected with meat, developed rinderpest, their temperatures rising on the third, fourth or fifth day after inoculation. Hill bulls 7252 and 7165 did not react, but both were found to be immune when tested with virulent blood.

Blood from 7192 stored at room temperature without protection remained infective for nine days. Meat from the same animal remained infective for five days and bone marrow for nine days. The materials were not tested at longer intervals.

EXPERIMENT 3.

1916, Sept. 23rd	Hill bull 7257, sixth day of rinderpest attack, vesicles in mouth and diarrhœa, bled to death. Blood kept in open dish at room temperature.
„ 25th (2 days)	Hill bull 7184 injected with 1 c.c. blood.
„ 26th (3 days)	„ „ 7176 „ „ „ „
„ 27th (4 days)	„ „ 7226 „ „ „ „
„ 28th (5 days)	„ „ 7267 „ „ „ „
„ 30th (7 days)	„ „ 7312 „ „ „ „
Oct. 2nd (9 days)	„ „ 7325 „ „ „ „

The air temperature varied from 54° to 69° F. Putrefaction was marked by the third day. On the ninth day the blood was almost desiccated.

Result. Hill bulls 7184, 7176, 7226, 7267, and 7312 contracted rinderpest. The temperatures of the first four bulls rose on the fourth day after injection and that of 7312 on the fifth day. 7325 did not become infected but was susceptible. Blood from 7257 exposed at air temperature remained infective for seven days but had lost its infectiveness after nine days' exposure.

EXPERIMENT 4.

1916, Sept. 27th	Hill bull 7223, sixth day of rinderpest attack, vesicles in the mouth, bled to death. Leg bones removed and kept exposed at room temperature.
„ 30th (3 days)	Hill bull 7311 injected with 5 c.c. extract of the bone marrow.
Oct. 3rd (6 days)	Hill bull 7165 „ „ 6 c.c. „
„ 6th (9 days)	„ „ 7346 „ „ 5 c.c. „
„ 12th (15 days)	„ „ 7322 „ „ 5 c.c. „

The air temperature varied from 52° to 69° F. Putrefactive organisms were present in the marrow at three days and at six days the material was quite putrid.

Result. Hill bulls 7311 and 7346 contracted rinderpest, their temperatures rising on the third day after inoculation. Bulls 7165 and 7322 failed to react but on testing with virulent blood 7165 was found to be immune while 7322 was susceptible. In bones from 7273 exposed at air temperature, the marrow was found to be infective up to 9 days after the death of the animal but not after 15 days.

EXPERIMENT 5.

1916, Oct. 10th	..	..	Hill bull 7338, fifth day of rinderpest attack, showing vesicles in the mouth and diarrhœa, bled and blood kept in open dish at air temperature.
,, 13th (3 days)	..	..	Hill bull 7373 injected with 1 c.c. blood.
,, 15th (5 days)	..	..	,, ,, 7378 ,, ,, 1 c.c. ,,
,, 17th (7 days)	..	..	,, ,, 7345 ,, ,, 1 c.c. ,,
,, 19th (9 days)	..	..	,, ,, 7395 ,, ,, 1 c.c. ,,

The air temperature varied from 49° to 64° F. Putrefaction of the blood was marked after the third day and by the ninth day the material was almost desiccated.

Result. The four test animals contracted rinderpest, their temperatures rising on the fourth and fifth days after inoculation but 7395 showed a very mild reaction and recovered. Inoculations after storage for longer intervals were not carried out. Blood from 7338 exposed at air temperature remained infective for 9 days.

EXPERIMENT 6.

1916, Dec. 1st, 11 A.M.			..	..	Hill bull 7590, fifth day of rinderpest attack, showing vesicles in the mouth and diarrhœa, bled and blood kept in an open dish at air temperature.			
,, 4th	,,	(3 days)	..	Hill bull 7618 injected 1 c.c. blood.				
,, 7th	,,	(6 days)	..	,,	,,	7637	,, 1 c.c.	,,
,, 10th	,,	(9 days)	..	,,	,,	7653	,, 1 c.c.	,,
,, 13th	,,	(12 days)	..	,,	,,	7668	,, 1 c.c.	,,
,, 16th	,,	(15 days)	..	,,	,,	7677	,, 1 c.c.	,,
,, 19th	,,	(18 days)	..	,,	,,	7683	,, 1 c.c.	,,
,, 22nd	,,	(21 days)	..	,,	,,	7809	,, 1 c.c.	,,
,, 25th	,,	(24 days)	..	,,	,,	7823	,, 1 c.c.	,,
,, 28th	,,	(27 days)	..	,,	,,	7846	,, 1 c.c.	,,
,, 31st	,,	(30 days)	..	,,	,,	7916	,, 1 c.c.	,,
1917, Jan. 3rd	,,	(33 days)	..	,,	,,	9	,, 1 c.c.	,,
,, 6th	,,	(36 days)	..	,,	,,	14	,, 1 c.c.	,,
,, 9th	,,	(39 days)	..	,,	,,	27	,, 1 c.c.	,,
,, 12th	,,	(42 days)	..	,,	,,	35	,, 1 c.c.	,,
,, 15th	,,	(45 days)	..	,,	,,	49	,, 1 c.c.	,,
,, 18th	,,	(48 days)	..	,,	,,	45	,, 1 c.c.	,,
,, 21st	,,	(51 days)	..	,,	,,	33	,, 1 c.c.	,,

1917, Jan.	27th	11 A.M.	(57 days)	..	Hill bull	31	injected	1 c.c.	blood.
Feb.	2nd	„	(63 days)	..	„ „	36	„	1 c.c.	„
„	8th	„	(69 days)	..	„ „	28	„	1 c.c.	„
„	14th	„	(75 days)	..	„ „	166	„	1 c.c.	„
„	20th	„	(81 days)	..	„ „	170	„	1 c.c.	„

The daily maximum shade temperature throughout this experiment varied from 46° to 56° F. and the minimum from 30° to 42° F.

After three days' storage the blood contained numerous putrefactive organisms and by the sixth day was smelling putrid ; owing to evaporation the blood became semi-solid by the 15th day and had to be diluted with saline solution before injection. By the 21st day the blood was quite solid in a layer about half an inch thick and almost desiccated, it was then ground up with saline solution in a mortar and the fluid injected ; on the 30th day the blood was completely desiccated.

Result. Hill bulls 7618, 7637, 7653, 7668, 7677, 7683, 7809, 7823, 7846, 7916, 9, 14, 27, 38, 49, 45, and 33 contracted rinderpest, their temperatures rising on the third or fourth day after inoculation. Hill bulls 31, 36, 28, 166, and 170 failed to become infected but were susceptible. Blood from 7590 exposed at air temperature remained infective for 51 days ; after 57 days' exposure it had become non-infective.

SUMMARY.

Experiment	Hill bull	Material taken	Intervals of exposure at air temperature after which tested	Result
1	7108	Blood ..	3 and 6 days ..	Not infective.
		Meat ..	3, 6 and 10 days ..	Infective after 3 days but not after 6 and 10 days.
2	7192	Blood ..	2, 3, 4 and 9 days ..	Infective after all intervals.
		Bone marrow	5 and 9 days ..	Infective after both intervals.
		Meat ..	3, 5 and 9 days ..	Infective after 3 and 5 days; test animal injected with 9 days' old meat found to be immune.
3	7257	Blood ..	2, 3, 4, 5, 7 and 9 days ..	Infective at all intervals up to 7 days, but not infective after 9 days.
4	7273	Bone marrow	3, 6, 9 and 15 days ..	Infective after 3 and 9 days, not infective after 15 days. Test animal injected with 6 days' old marrow found to be immune.
5	7338	Blood ..	3, 5, 7 and 9 days ..	Infective at all intervals.
6	7590	Blood ..	3, 6, 9, 12, 15, 16, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 57, 63, 69, 75 and 81 days.	Infective at all intervals up to 51 days, but not infective after 57 days.

Conclusions.

(1) The length of time that the rinderpest virus is able to survive in blood from a sick animal kept at air temperature in an open vessel varies within wide limits ; in one observation such blood was non-infective after 3 days' exposure, while in another it remained infective for 51 days although putrefaction set in after a few days' exposure and by the 30th day the blood was completely desiccated. In two other observations blood was still infective after 9 days' exposure to the air and in a third it was infective after 7 days' but non-infective after 9 days' exposure.

(2) In two observations the virus maintained its vitality in bone marrow for 9 days, but in one of these cases infectiveness was lost after 15 days.

(3) Meat was infective after 3 days in one observation when blood from the same animal was non-infective within that period ; in another case meat remained infective for 5 days.

(4) Further tests are necessary to determine the factors influencing the survival of the rinderpest virus in animal tissues under natural conditions ; the temperature at which the material is kept appears to have a considerable effect, possibly in determining the rate and character of the putrefactive changes taking place, but it has been shown that these may not destroy the virus as rapidly as many authorities have stated to be the case.

VI. GENERAL CONCLUSIONS.

(1) At the Muktesar Laboratory, rinderpest infection was found to persist in certain buildings for 48 hours after the removal of the sick animals but not for longer periods of time; frequently infection was absent after shorter intervals. Ground which was shaded by trees, when contaminated by cattle suffering from rinderpest, was found to be infective to healthy stock 18 hours after the removal of the sick animals but not after longer intervals. Ground entirely exposed to direct sunlight did not remain infective beyond 8 hours.

(2) In the plains buildings were found to remain infective for 20 hours after removal of the sick animals but were non-infective after longer intervals. Areas shaded by trees remained infective for 24 hours and those exposed to direct sunlight for 6 hours after removal of the sick animals, but not for longer periods of time.

(3) The rinderpest virus was found to survive in mixed fæces and urine protected from direct sunlight for periods up to 54 hours after excretion by sick animals, but when exposed to sunlight the virus did not survive for longer periods than 8 hours. Saliva and nasal discharge from sick animals did not remain infective beyond 44 hours.

(4) It may be concluded therefore that in buildings and on areas infected by the natural discharges of sick animals, the rinderpest virus is unable to survive for more than two or three days, and when air and sunlight are freely admitted its destruction is even more rapid.

(5) The carcases of animals which have died from the disease must, however, be regarded as possible sources of infection for some considerable time after death, especially when the air temperature is low, as it has been shown that the virus can survive for 51 days in blood from a sick animal even when this has been freely exposed to the air and allowed to become putrid[1]; in meat and bones also the virus may persist for many days.

Further observations are necessary to determine the factors influencing the duration of the vitality of the rinderpest virus in dead animal tissues.

[1] In view of the statements made by various authorities to the effect that the rinderpest virus will not survive in drawn blood for more than a day or two, it may be mentioned that in a very large number of observations carried out at the Muktesar Laboratory, defibrinated blood stored under aseptic conditions at room temperature remained virulent for periods of from 30 to 40 days, and in one case blood kept at 0°C. was virulent after 90 days.

VIRULENCE OF TUBERCLE BACILLI ISOLATED FROM BOVINE LESIONS IN INDIA.

BY

A. L. SHEATHER, B.Sc., M.R.C.V.S.,

Director and First Bacteriologist, Imperial Bacteriological Laboratory, Muktesar.

[Received for publication on 6th April, 1920.]

THE literature referring to the occurrence of tuberculosis in cattle in India is very scanty, and the whole of it has been published during the last twenty years or so.

The most recent additions to it are articles by Glen Liston and Soparkar, and by Taylor, the former in the *Indian Journal of Medical Research* (Vol. V, No. 1, July 1917, pp. 19 to 71) and the latter in the same Journal (Vol. V, No. 3, January 1918, p. 497).

The published records would appear to indicate that bovine tuberculosis is distinctly uncommon in India, but Taylor's investigations throw a rather different light on the subject, at least so far as the South Punjab is concerned.

Taylor found, in the course of systematic inspections at the Ferozepore slaughter-houses, that about 3 per cent. of the animals *actually slaughtered* had lesions of tuberculosis. Special attention is drawn to the fact that all animals have to be inspected prior to slaughter, and that nearly 18 per cent. of the whole number were rejected on account of their poor condition. It is, therefore, reasonable to assume that 3 per cent. does not represent the actual number of tuberculous animals, as doubtless many of those rejected prior to slaughter were in poor condition owing to infection with tuberculosis.

The materials upon which the present investigation is based were obtained from some of the specimens sent by Mr. Taylor to Muktesar for examination.

A few attempts were made to obtain primary cultures direct from the specimen by using antiformin. These were, however, abandoned, as satisfactory results were not obtained, owing largely to the difficulty of obtaining good samples of bleaching powder for the preparation of the antiformin.

It may be here noted that a systematic examination of the cultural characters of the bacilli isolated could not be carried out, because pressure of other work did not permit it.

It is hoped that in the future time will be available for this.

Materials from 16 cases were used for the inoculation of small animals. Details of the lesions found in the carcases at Ferozepore were not supplied with the earlier specimens, but at my request Mr. Taylor kindly supplied details of the last eight.

These were as follows:—

TABLE I.

No. & date	Breed	Sex	Age	Lesions	Micro. exam.
142 / 8-5-17	Local	Bull	7	Caseo-calcareous tubercles in anterior lobes of both lungs. Bronchial and mediastinal glands greatly enlarged and full of caseo-calcareous tubercles.	++++
219 / 16-5-17	,,	Cow	6	A calcareous lesion in one bronchial gland.	++
504 / 13-2-18	,,	,,	6	Complete replacement of one mediastinal gland by calcification and half the other calcified.	++
526 / 21-2-18	,,	,,	4	Mediastinal glands greatly enlarged and almost completely caseous.	+
527 / 21-2-18	,,	,,	6	Caseation of one bronchial gland.	+++
529 / 25-2-18	,,	Bullock	7	Mediastinal gland completely calcified.	—
530 / 25-2-18	,,	Cow	5	Calcereous nodules in one bronchial gland.	++
531 / 2-3-18	,,	,,	5	Both bronchial glands calcareous.	+++

++++ = Bacilli numerous.
+++ = Bacilli easily detected.
++ = Bacilli scanty.
+ = Bacilli found after prolonged search.

Details of animals inoculated.

Specimen 116. The pus was subjected to the action of 20 per cent. antiformin for half an hour.

One guinea-pig was injected subcutaneously and one intraperitoneally with 0·5 c.c. of a suspension of the sediment in salt solution.

Guinea-pig 1863. Subcutaneous. Lived 157 days. Extensive tuberculosis of liver, spleen, lumbar, and right precrural glands.

Guinea-pig 1864. Intraperitoneal. Lived 113 days. Extensive tuberculosis of liver, spleen, lungs and omentum.

Specimen 117. Treated as No. 116.

Guinea-pig 1865. Subcutaneous. Lived 93 days. Extensive tuberculosis of the liver and spleen, two small tubercles in lungs. Precrural gland caseous.

Guinea-pig 1866. Intraperitoneal. Lived 115 days. Extensive tuberculosis of liver, spleen, lungs, and omentum.

Specimen 118. Treated as No. 116.

Guinea-pig 1867. Subcutaneous. Lived 217 days. Extensive tuberculosis of liver and spleen. Precrural gland caseous.

Guinea pig 1868. Intraperitoneal. Lived 107 days. Tuberculosis of liver, spleen and omentum. A few well-defined tubercles in lungs.

Specimen 122. Gland emulsion treated with antiformin. Dose 1 c.c. of suspension of sediment.

Guinea-pig 1869. Subcutaneous. Lived 152 days. Extensive tuberculosis of liver, spleen, and lumbar glands. Precrural gland caseous.

Guinea-pig 1870. Intraperitoneal. Lived 73 days. Extensive tuberculosis of liver, spleen, omentum, and lungs.

Specimen 123. Emulsion of gland treated with antiformin. Dose 1 c.c. of suspension of sediment.

Guinea-pig 1871. Subcutaneous. Lived 89 days. Spleen immensely enlarged and tuberculous. Extensive tuberculosis of abdominal glands and liver.

Guinea-pig 1872. Died of peritonitis following puncture of intestine.

Specimen 126. Emulsion of gland treated with antiformin. Dose 1 c.c. of suspension of sediment.

Guinea-pig 1874. Subcutaneous. Lived 84 days. Appears to have been injured, possibly by handling. All organs normal.

Guinea-pig 1875. Subcutaneous. Killed after 357 days, healthy and fat.

Guinea-pig 1873. Intraperitoneal. Died on the 13th day. Very poor condition. No lesions.

Guinea-pig 1876. Intraperitoneal. Died on the 7th day. Very poor condition.

Specimen 128. Emulsion of gland treated with antiformin. Dose 1 c.c. of suspension of sediment.

Guinea-pig 1877. Subcutaneous. Lived 198 days. Extensive tuberculosis of liver, spleen, and abdominal glands.

Guinea-pig 1878. Intraperitoneal. Lived 126 days. Extensive tuberculosis of omentum, liver, spleen, lungs, and abdominal glands.

Specimen 129. Emulsion of gland treated with antiformin. Dose 1 c.c. of suspension of sediment.

Guinea-pig 1879. Subcutaneous. Died on 7th day. Very poor condition. No lesions.

Guinea-pig 1880. Intraperitoneal. Died on 3rd day. Very poor condition.

Specimen 142. Emulsion of gland treated with antiformin. Dose 1 c.c.

Rabbit 1421. Subcutaneous. Killed on the 273rd day. Perfectly healthy.

Rabbit 1422. Intraperitoneal. Killed on 291st day. Lungs closely beset with tubercles as large as pins' heads. In these tubercles bacilli were present in moderate numbers. Kidneys beset with pin-head tubercles. Smears from these lesions shewed five or six bacilli in every field. One pin-head tubercle in omentum.

Guinea-pig 1904. Subcutaneous. Killed on 269th day. Perfectly healthy.

Guinea-pig 1905. Intraperitoneal. Killed on the 271st day. One pin-head tubercle in left lung. Spleen about five times normal size, containing nine or ten nodular lesions varying in size up to that of a pea. These nodules had the appearance of being composed of agglomerations of minute, translucent tubercles. Examination of preparations from this spleen shewed tubercle bacilli in small numbers. An emulsion of a piece of this spleen was used for the inoculation of another guinea-pig (No. 2012).

Guinea-pig 2012. 1 c.c. spleen emulsion from Guinea-pig 1905 intraperitoneally. Died on 116th day. A caseating lesion as large as a vetch at the point of penetration of the needle. Liver greatly enlarged and presenting the typical, irregular, yellow patches. Omentum contracted and sausage-like. Spleen eight to ten times the normal size and almost entirely necrotic. Mesenteric glands as large as peas and caseous. Lungs crowded with pin-head tubercles.

Specimen 219. Gland emulsion treated with antiformin. Dose 1 c.c.

Guinea-pig 1906. Intraperitoneal. Killed on 266th day. Perfectly healthy.

Rabbit 1424. Intraperitoneal. Killed on 286th day. Perfectly healthy.

Specimen 504. Gland emulsion without antiformin. Dose 1 c.c.

Guinea-pig 2015. Intraperitoneal. Died on 105th day. Caseating lesion as large as a horse-bean at the point of penetration of the needle. Liver greatly enlarged and beset with irregular yellow, necrotic patches. Spleen about six times the normal size and crammed with pin-head tubercles. Omentum shrunken and beset with tubercles.

Guinea-pig 2016. Intraperitoneal. Died on 49th day. Carcase destroyed in error before examination was made.

Specimen 526. Gland emulsion. Dose 0·5 c.c.

Guinea-pig 2029. Subcutaneous. Died on 5th day. Cause of death not determined.

Guinea-pig 2030. Subcutaneous. Died on 5th day. Cause of death not determined.

Guinea-pig 2031. Subcutaneous. For this and the next inoculation the material was treated with antiformin. Died on 194th day. Caseous lesion at the seat of inoculation, precrural gland enlarged and congested. Liver enlarged and mottled in colour, but no definite areas of necrosis. Spleen enlarged, about a score of pin-head tubercles. Lungs, about a score of pin-head tubercles.

Guinea-pig 2032. Intraperitoneal. Died on 91st day. Liver enlarged with numerous typical necrotic patches. Spleen about five times the normal size, contained about a dozen tubercles varying in size from a pea downwards. Omentum partly contracted and beset with small tubercles.

Specimen 527. Gland emulsion treated with antiformin. Dose 1 c.c.

Guinea-pig 2033. Intraperitoneal. Died on 124th day. Liver markedly cirrhotic. Three irregular necrotic patches about as large as peas. Spleen about ten times the normal size, and beset with tubercles. Omentum contained four or five caseous tubercles varying in size from a pea downwards.

Guinea-pig 2034. Subcutaneous. Died on 153rd day. Small, caseous lesion at the seat of inoculation. Liver greatly enlarged and to a large extent necrotic. Spleen markedly tuberculous. A few small tubercles in the right lung.

Specimen 529. Emulsion of gland treated with antiformin. Dose 0·5 c.c.

Guinea-pig 2039. Subcutaneous. Died on 3rd day. Cause of death not determined.

Guinea-pig 2040. Intraperitoneal. Died on 183rd day. Liver about twice normal size, markedly cirrhotic and containing three or four necrotic patches as large as peas. Spleen greatly enlarged and beset with confluent tuberculous patches. Lungs crowded with small tubercles. Omentum partly shrunken and containing caseous tubercles.

Specimen 530. Gland emulsion treated with antiformin. Dose 0·5 c.c.

Guinea-pig 2041. Subcutaneous. Died on 199th day (during my absence from Muktesar). No post-mortem notes were made, but the animal was infected as cultures were obtained.

Guinea-pig 2042. Intraperitoneal. Died on 126th day. Liver greatly enlarged and almost entirely necrotic. Omentum completely shrunken and beset with tubercles. Spleen greatly enlarged and beset with tubercles. Anterior and middle lobes of both lungs containing numerous tubercles. Sternal glands enlarged and caseous.

Specimen 531. Emulsion of gland treated with antiformin. Dose 0·5 c.c.

Guinea-pig 2043. Subcutaneous. Died on 145th day. No evidence of tuberculosis.

Guinea-pig 2044. Intraperitoneal. Died on 57th day. No evidence of tuberculosis.

With the exception of No. 529 all the specimens from which inoculations were made contained tubercle bacilli in sufficient numbers to render their detection possible by microscopic examination. The results of inoculations made with specimen 529 proved that they were present in that also.

Cases 126, 129, 219, and 531 require special mention.

In No. 126 the two guinea-pigs inoculated intraperitoneally died very soon after inoculation. Of the two done subcutaneously, one died apparently as the result of an accident after 84 days. Had living bacilli been present in the material used this animal might have been expected to show lesions. The remaining one was killed nearly a year after inoculation and was found to be healthy. Bacilli had been detected in the original material with the microscope. It, therefore, appears that although the bacilli were recognisable, they were actually dead or at least incapable of causing infection.

Both the guinea-pigs inoculated from specimen 129 died too soon after inoculation to allow of the development of lesions.

The carcase from which specimen 219 was taken contained only a calcareous lesion in one bronchial gland. Bacilli were detected microscopically, but a guinea-pig and a rabbit inoculated intraperitoneally failed to become infected. They were killed on the 266th and 286th days, respectively.

As the lesion was markedly calcified it is probable that the bacilli contained in it were dead although still visible. A similar explanation possibly holds good for the failure to infect guinea-pigs 2043 and 2044 from case 531. These animals died on the 145th and 57th days, and might have been expected to shew some evidence of infection had the bacilli contained in the original material been viable.

Excluding the foregoing and all animals which, although inoculated with material proved to be infective by the results obtained in other animals inoculated with the same material, died before the development of lesions, there remain for criticism the results obtained in 10 guinea-pigs inoculated subcutaneously, 11 guinea-pigs inoculated intraperitoneally, one rabbit inoculated subcutaneously, and one intraperitoneally.

The duration of life in the guinea-pigs inoculated subcutaneously ranged from 89 to 217 days. One guinea-pig, No. 1904, hardly falls into the group mentioned above of those which, although inoculated with infective material, died before lesions had time to develop, as it was kept under observation for 269 days and was then found to be healthy on post-mortem examination. That the material was infective is proved by the results obtained in the fellow guinea-pig, No. 1905. The extraordinary length of life of guinea-pig 1905 would appear to suggest either that the particular bacilli present in the original material were of very low virulence, or that only a minimum number of those present were viable. The latter appears to be the probable explanation, the case forming a connecting link between those in which what may be called normal results followed the inoculations and those in which, although tubercle bacilli were recognisable in the seed material, no lesions were produced in inoculated animals.

The duration of life of the guinea-pigs inoculated intraperitoneally ranged from 73 to 183 days. One guinea-pig, No. 1905, to which reference has just been made, is excluded from this statement. This animal was killed after 271 days and was found to be infected. The nature of the infection in guinea-pig 1905 was definitely settled by the inoculation of guinea-pig 2012.

From the Report of the Royal Commission on Tuberculosis, Part II, Appendix, Vol. 1, p. 473, it may be gathered that emulsions of tuberculous tissues (of European bovine origin) when inoculated subcutaneously into guinea-pigs caused death in from 27 to 146 days, and when given intraperitoneally in from 8 to 140 days.

A comparison of these figures with those recorded in this paper indicates that tubercle bacilli of Indian origin are distinctly less virulent than those of European origin.

Pressure of other work rendered it impossible to undertake a systematic examination of the cultural characters of the various strains, but generally speaking they possessed the usual characters of bovine bacilli and were distinctly dysgonic.

After subcultivation upon egg and serum agar for some generations a few strains were induced to grow upon the surface of glycerin broth. Growth was

very slow indeed. The islands tended to spread until they measured an inch or so in diameter, but after that lateral growth stopped and some degree of thickening occurred.

One of the strains giving the best growths upon broth was that obtained from case 118 isolated through guinea-pig 1868.

Primary cultures were obtained on egg medium, and from subcultures upon serum agar the 9th generation was induced to grow on broth.

The 10th generation which was also grown upon broth was used for the inoculation of buffalo and bull calves.

Several flasks of this generation had been in the incubator for six months. In each flask there was an island of growth an inch or so in diameter. These islands were dry-looking and rather thick.

To obtain sufficient culture for the experiments the growth was lifted out of a number of the flasks and placed in the upper part of wide tubes which were then placed in a nearly vertical position to drain away as much of the broth as possible. The following day the growth was placed upon sterile filter paper in petri dishes. In this way practically all the free liquid was removed.

A sufficient quantity of this growth was thoroughly rubbed up in a mortar with 0·4 per cent. salt solution so that 1 c.c. of the emulsion contained 5 milligrammes of bacilli (Emulsion A).

One cubic centimetre of this emulsion was diluted with 49 c.c. of the salt solution. This emulsion represented 0·1 mg. per cubic centimetre (Emulsion B).

One cubic centimetre of emulsion B was diluted with 9 c.c. of the salt solution. The bacterial content of this emulsion (Emulsion C) was 0·01 mg. per ubic centimetre.

Two rabbits, Nos. 60A and 62A, were inoculated intravenously with 1 c.c. of Emulsion B, and two others, Nos. 76 and 77, with 1 c.c. of Emulsion C.

Rabbit 60A died on the 43rd day of acute, generalised tuberculosis, lungs, liver, spleen and kidney all containing miliary lesions.

Rabbit 62A died on the 36th day. The lungs were crowded with tubercles varying in size from a pin's head to a lentil. The spleen contained about a dozen pin-head tubercles. There were no visible lesions elsewhere.

Rabbit 76 died on the 50th day of generalised tuberculosis, lesions being present in the lungs, liver, spleen and kidneys.

Rabbit 77 died on the 70th day with similar lesions.

From the Report of the Royal Commission on Tuberculosis, Part II, Appendix, Vol. 1, p. 437, it may be gathered that rabbits inoculated intra-

venously with 0·1 mg. of culture invariably died of generalised tuberculosis within 34 days. The average period was 21 days.

The fact that the control rabbits used in the present experiments which received the same dose lived about twice as long is an index of the lower virulence of the virus used.

Experiments with cattle.

For the experiments with cattle thirteen plains bull calves and thirteen plains buffalo calves from eight to twelve months old were obtained from the Bareilly District. It was found impossible to obtain younger animals as the villagers would not dispose of them.

It was intended that twelve of each of these animals should be inoculated subcutaneously with tubercle bacilli, half of each group with 10 mg. and the other half with 50 mg. The thirteenth animal of each kind was to remain as a control.

Two of the buffaloes, Nos. 225 and 226, died shortly before the experiment started and, as it would have taken ten days or possibly a fortnight to replace them, it was decided to carry on the experiment with the animals available.

The whole of them were first subjected to a tuberculin test on October 8th, 1919. Three cubic centimetres of tuberculin were injected subcutaneously and 0·25 c.c. of concentrated tuberculin (100-20) was injected into the skin of the left lower eyelid. The temperature of buffalo 219 rose from 102·5° at the time of injection to 105° at the 18th hour. Examination of the blood shewed, however, that trypanosomes were present in large numbers. As experience has shewn in innumerable cases that the appearance of trypanosome in the blood of an apparently normal animal is almost invariably accompanied by a rise of temperature, the rise in this case was ignored as far as the tuberculin test was concerned.

The temperature of buffalo 221 was very irregular during the period of the test. At the time of injection it was 101°. It was 102° at the 9th hour. Fell to 99·5° by the 15th hour and then rose rapidly to 105° by the 24th hour. During the next 36 hours it fell rapidly and regularly to 98·5°.

Examination of the blood failed to reveal any cause for this. The rise was not considered to be sufficiently regular to indicate a positive reaction, more especially as the temperature had been far from regular for more than a month. This is not uncommonly seen in buffaloes. None of the other buffaloes shewed any rise of temperature. None of the bull calves shewed any reaction whatever.

It may be here stated that the value of the ophthalmic and intradermalpalpebral tests is practically nil, at least so far as concerns plains buffaloes

brought to the hills, as they are extremely liable to catarrhal conjunctivitis at all times. In the case of this test any discharge present was ignored as the uninjected eyes varied very considerably from hour to hour during the test.

The buffaloes and calves were each divided into two groups, so that individuals in the groups were, respectively, as nearly as possible pairs as far as their weight was concerned. The odd buffalo and calf were kept as controls.

One group of buffaloes and one group of calves were then inoculated subcutaneously on the left side of the neck with emulsion of tubercle bacilli (Emulsion A), the dose being 10 mg. The other groups were inoculated in the same way with 50 mg.

The following table gives the numbers of the animals, age, weight, dose of tubercle bacilli, and date of inoculation.

TABLE II.

Animal	Age	Weight	Inoculation		Date
		lb.	mg.		
Buff. 215 ..	1 year	210	10	subcut.	11-10-19
,, 217 ..	,,	255	,,	,,	,,
,, 219 ..	,,	260	,,	,,	,,
,, 222 ..	,,	300	,,	,,	,,
,, 220 ..	,,	330	,,	,,	,,
,, 223 ..	,,	225	50	,,	,,
,, 224 ..	,,	255	,,	,,	,,
,, 218 ..	,,	270	,,	,,	,,
,, 216 ..	,,	280	,,	,,	,,
,, 227 ..	,,	315	,,	,,	,,
,, 221 (control) ..	,,	238	Not inoculated		
			mg.		
B. calf 9 ..	10 months	140	10	,,	,,
,, ,, 3 ..	8 ,,	152	,,	,,	,,
,, ,, 1 ..	1 year	170	,,	,,	,,
,, ,, 2 ..	,,	235	,,	,,	,,
,, ,, 6 ..	,,	245	,,	,,	,,
,, ,, 5 ..	,,	275	,,	,,	,,
,, ,, 13 ..	,,	140	50	,,	,,
,, ,, 4 ..	,,	160	,,	,,	,,
,, ,, 11 ..	,,	200	,,	,,	,,
,, ,, 8 ..	,,	220	,,	,,	,,
,, ,, 10 ..	,,	245	,,	,,	,,
,, ,, 12 ..	,,	310	,,	,,	
,, ,, 7 (control) ..	,,	360	Not inoculated		

On November 16th, one month after inoculation with the tubercle bacilli, the animals were subjected to a second tuberculin test. On this occasion 3 c.c. of tuberculin was the dose used for the subcutaneous test as before, and 0·25 c.c. of concentrated tuberculin was injected into the right anal fold of all the buffaloes and of calves 2, 3, 5, 6, 12, and 13. The remaining calves 1, 4, 8, 9, 10, 11, and 7 received 0·25 c.c. into the right lower eyelid.

The tuberculin was injected at 9 P.M. and the temperatures were taken at intervals of three hours from the 9th to the 24th inclusive.

Before attempting to assess the value of the temperature reactions as indicators of actual infection with tubercle bacilli, a conclusion must be arrived at as to what extent the temperature must rise to constitute a positive reaction, and, further, what temperature must be taken as the basis when the rise is being calculated. The experiments of McFadyean and Sheather (*Journal of Comparative Pathology and Therapeutics*, Vol. XXV, 1914, pp. 323-388) shewed that in the great majority of cases a rise of 2° above the temperature at the time of the injection of the tuberculin constituted a reaction. The results were far more accurate when this standard was adopted than when, as has been suggested should be done, the rise of temperature was calculated from the maximum temperature recorded on the day prior to the test, the temperature being taken at least twice, morning and evening, on that day.

In assessing the results recorded here the standard adopted is that a rise of 2° or more above the temperature at the time of injection will be considered as a positive seaction.

The following table gives the temperatures recorded during the test, and in addition the morning and afternoon temperatures on the day of injection of the tuberculin.

TABLE III.

Animal	10–11–19			11–11–19						Result
	10 A.M.	3 P.M.	9 P.M.	9th	12th	15th	18th	21st	24th	
Buff. 215 ..	100·4	101·2	101·4	104·0	104·0	105·2	103·4	102·4	102·4	+
„ 217 ..	101·2	100·4	102·2	106·0	105·4	105·2	104·8	105·6	105·2	+
„ 219 ..	101·4	102·2	101·2	106·2	105·6	104·4	103·2	102·8	102·2	+
„ 222 ..	101·2	101·2	101·6	103·2	104·2	104·4	105·2	104·2	103·2	+
„ 220 ..	100·2	101·2	101·8	103·0	103·2	104·8	104·8	105·2	105·6	+
„ 223 ..	101·2	100·4	100·8	102·2	100·8	102·0	104·2	103·6	102·0	+
„ 224 ..	99·8	100·4	101·6	104·0	104·0	103·6	105·0	104·0	105·0	+
„ 218 ..	101·4	101·4	101·6	104·0	103·2	103·0	103·6	104·4	104·0	+
„ 216 ..	100·0	102·0	101·0	104·2	104·4	103·6	104·2	105·0	104·6	+
„ 227 ..	101·8	102·4	101·6	104·4	105·2	104·2	104·0	104·0	103·4	+
„ 221 .. (control)	100·0	101·6	101·0	100·0	101·0	100·6	101·0	101·4	101·4	—
B. calf 9 ..	102·4	103·0	101·8	102·2	102·8	102·6	103·0	103·0	103·0	—
„ „ 3 ..	101·6	102·4	101·6	102·6	102·8	103·2	103·2	102·4	103·2	—
„ „ 1 ..	101·2	102·2	102·0	103·2	103·4	103·0	104·2	104·2	104·2	+
„ „ 2 ..	102·0	101·4	99·8	102·0	102·8	104·2	103·4	103·6	103·2	+
„ „ 6 ..	101·2	102·0	101·4	104·0	104·0	104·2	104·2	104·0	104·0	+
„ „ 5 ..	101·2	102·0	102·0	104·0	104·0	103·8	103·0	103·8	103·4	+
„ „ 13 ..	101·6	102·4	102·0	103·4	103·0	103·2	104·4	102·2	103·0	+
„ „ 4 ..	101·2	102·8	101·6	103·0	102·8	103·0	103·6	103·0	103·2	+
„ „ 11 ..	101·6	103·2	102·0	102·0	102·0	103·2	103·8	103·4	103·2	—
„ „ 8 ..	101·6	102·4	101·2	102·0	103·6	103·2	103·2	103·2	102·4	+
„ „ 10 ..	102·4	102·2	102·0	103·2	104·0	104·2	104·2	104·0	104·0	+
„ „ 12 ..	101·6	102·4	102·0	102·0	102·2	101·8	103·0	102·4	103·0	—
„ „ 7 .. (control)	100·6	102·0	100·8	100·4	102·0	101·2	101·4	101·8	101·4	—

Considering the buffaloes first it will be seen that none of them had an abnormal temperature prior to the injection of the tuberculin. The highest temperature was 102·4° (No. 227).

This temperature, however, is very commonly recorded in normal buffaloes here.

Taking a rise of 2° above the temperature at the time of injection as indicating a positive reaction it will be seen that all the infected buffaloes reacted. The rises ranged from 2·8° to 5°. The hour at which the maximum temperature was recorded varied very considerably. In some cases it was at the 9th and in others as late as the 24th.

The temperature of buffalo 217 did not return to normal until the second day after the injection of the tuberculin.

Buffalo 221 (control) shewed a maximum rise of 0·4° only.

The temperatures of the bull calves prior to injection were rather more irregular than those of the buffaloes. Irregularity of temperature is, however, very common and temperatures up to 103° are not infrequently recorded in apparently healthy animals. At the time of injection the temperature in no case exceeded 102°.

Applying the standard suggested it is found that eight shewed rises of 2° or more, and four from 1° to 1·8°. The reactions of the bull calves are, therefore, far less marked than those of the buffaloes both in number and extent.

Special mention must be made of the extraordinarily marked local reactions which developed at the seat of injection of the subcutaneous dose of tuberculin on the side of the neck in some of the animals. These bore a very close resemblance to typical local reactions to mallein. Details of the reactions shewn by the anal folds are also given.

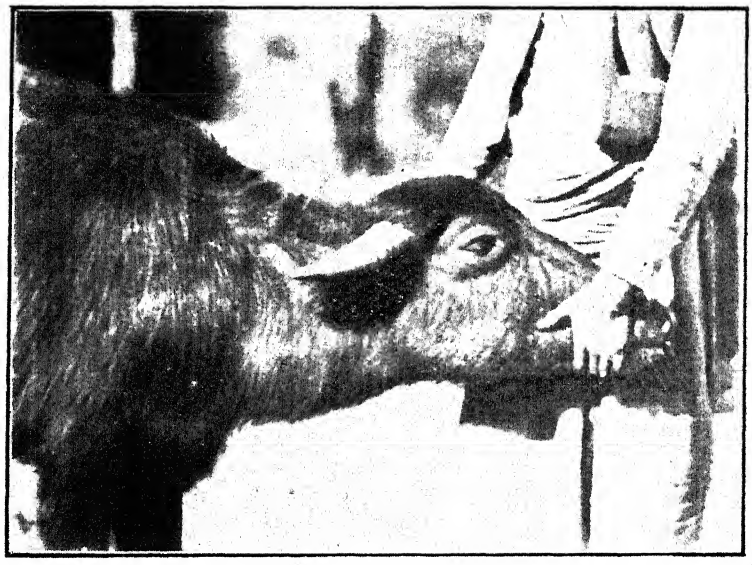
Buffalo 215. Painful swelling 4 inches in diameter at the 9th hour persisting in this form up to the 18th hour. Slight swelling for four days after the test.

Just appreciable thickening of the anal fold up to the 24th hour only.

Buffalo 217. Painful swelling 7 inches by 6 at the 9th hour persisting up to the 36th hour. The swelling steadily decreased and disappeared on the 4th day.

Anal fold hard and twice the normal thickness up to the 24th hour. Thickening disappeared on the following day.

Buffalo 219. Painful swelling which was slight at the 9th hour, but which attained the size of an orange by the 24th hour. It then decreased and had disappeared by the 4th day.



Buffalo 220. Local reaction to the subcutaneous tuberculin test at the 39th hour. November 12th, 1919.

A slight thickening of the anal fold which had disappeared by the 24th hour.

Buffalo 222. Hot, painful swelling which attained the size of an orange by the 21st hour. It then slowly decreased and had not disappeared until the 6th day.

The anal fold was three times the normal thickness during the first 24 hours. The following day the thickening was only just appreciable. This condition persisted until the 6th day.

Buffalo 220. A hot, painful swelling which steadily increased up to the 24th hour when it measured 7 by 5 inches. On the following morning, 36 hours after injection, the swelling had become softer and more diffuse. From this time onwards the swelling became softer and smaller, finally disappearing on the 6th day. (Plate I.)

The anal fold was twice the normal thickness at the 18th hour. It then declined, but remained appreciable up to the evening of the 4th day.

Buffalo 223. A slight thickening on the neck which was only just appreciable after the first 24 hours.

The anal fold shewed a slight thickening which disappeared on the day following injection.

Buffalo 224. A hot, painful swelling on the neck which measured 7 by 5 inches at the 21st hour. This persisted without appreciable change until the morning of the second day after the test. It then steadily declined and had disappeared on the 5th day.

The anal fold attained a thickness of four times the normal by the 18th hour, it then declined and was just appreciable at the 36th hour.

Buffalo 218. Slight swelling on the neck which persisted for 36 hours.

The anal fold attained a maximum thickness of twice the normal during the first 24 hours. There was just appreciable thickening up to the 4th day.

Buffalo 216. Hot, painful swelling which attained the size of a coconut by the 18th hour. This persisted up to the 60th hour. It then steadily decreased in size and had disappeared on the 6th day.

The anal fold attained a maximum thickness of three times the normal at the 15th hour. The thickening was just appreciable at the 36th hour and remained practically unchanged up to the 4th day.

Buffalo 227. Hot, painful swelling on the neck which was as large as an orange by the 18th hour. It persisted without change up to the 48th hour, then decreased, and had disappeared by the 4th day.

The anal fold was three times the normal thickness at the 18th hour. From this time the thickening decreased and had quite disappeared on the 6th day.

Buffalo 221 (control). A slight swelling on the side of the neck which was neither hot nor painful. This had disappeared by the 15th hour.

The anal fold was just appreciably thickened during the first 24 hours only.

Bull calf 9. Hot, painful swelling in front of the shoulder which was as large as an orange by the 18th hour. This was maintained until the 24th hour. At the 36th hour it was slightly reduced and had disappeared by the 4th day.

The eyelid shewed slight swelling from the 9th to the 24th hour.

There was no discharge.

Bull calf 4. Slight swelling on the neck which disappeared within 36 hours. Slight swelling of the eyelid which disappeared in the same time.

Bull calf 11. Hot, painful swelling 3 inches across at the 9th hour which increased slightly up to the 24th hour. It then decreased but was still appreciable on the 4th day.

The eyelid was slightly swollen for 24 hours after injection and there was a mere trace of muco-purulent discharge at the 21st hour.

Bull calf 8. Slight swelling on the side of the neck which persisted for 48 hours. It was not hot or painful at any time.

No eye reaction beyond slight swelling of the lid for 24 hours.

Bull calf 10. Slight swelling of the neck, neither hot nor painful, which lasted for 48 hours.

Slight swelling of the eyelid which lasted for 24 hours.

Bull calf 7. Slight swelling of the neck which had disappeared by the 15th hour.

Slight swelling of the eyelid which had disappeared by the 15th hour.

Bull calf 3. Swelling on the neck about 3 inches in diameter at the 9th hour. This steadily decreased and had disappeared in 36 hours.

Slight thickening of the anal fold which had disappeared in 24 hours.

Bull calf 2. Hot, painful swelling on the neck as large as an orange. This persisted up to the 24th hour. At the 36th hour it was very much smaller and disappeared by the evening of the 3rd day.

The anal fold maintained a thickness of twice the normal up to the 24th hour, after which a mere trace of thickening could be felt up to the 3rd day.

Bull calf 6. A hot, painful swelling on the front of the shoulder at the 9th hour. The animal walked a little lame. The swelling persisted up to the

24th hour. At the 36th hour the swelling was only slight, it remained appreciable until the evening of the 3rd day.

Bull calf 5. A hot, painful swelling 3 inches by 2 in front of the shoulder. The animal was a little lame. This swelling persisted for 36 hours and then steadily decreased. None was appreciable on the morning of the 4th day.

The anal fold shewed a slight thickening during the first 36 hours only.

Bull calf 13. Slight swelling on the neck which persisted for 36 hours only.

The anal fold shewed a circumscribed, hard swelling like a marble from the 15th to the 36th hours. This had quite disappeared on the following day.

Bull calf 12. A hot, painful swelling in front of the shoulder 3 inches by 2. The animal was slightly lame. The swelling persisted up to the 24th hour. At the 36th hour there was only a slight amount of swelling. This, however, remained appreciable until the 4th day.

The anal fold was twice the normal thickness up to the 24th hour. The thickening was just appreciable until the evening of the 3rd day.

It is always a matter of difficulty to appraise the value of the intradermal tests unless they are very marked, and I am inclined to think that judgment upon the reactions obtained in the anal folds must be suspended until further experience has been obtained. It certainly is a fact that more marked reactions were shewn by practically every one of the infected animals than by the controls.

The eye reactions I consider as all negative.

It remains to consider what significance, if any, attaches to the pronounced reactions obtained at the seat of the subcutaneous injection of the tuberculin.

It must be admitted that when these swellings were observed it was hoped that it might prove to be a constant result in tuberculous animals, more especially as the controls shewed nothing approaching the reactions given by the infected cattle.

The possibility of the presence of some contamination in the tuberculin being responsible was considered, but the absence of such reactions in the controls appeared to negative this.

After the lapse of a month the whole of the animals were subjected to another test.

On this occasion the subcutaneous test was alone applied. The dose of tuberculin was 3 c.c.

The following table shows the temperatures recorded.

TABLE IV.

Animal	11–12–19			12–12–19						Result
	10A.M.	3P.M.	9P.M.	9th	12th	15th	18th	21st	24th	
Buff. 215 ..	98·4	101·2	100·8	101·8	101·8	101·0	101·2	103·0	101·9	+
„ 217 ..	100·2	100·8	101·6	103·0	104·0	103·6	103·2	104·4	102·0	+
„ 219 ..	102·0	101·0	102·2	104·0	104·0	103·8	103·4	104·0	103·0	—
„ 222 ..	100·6	101·2	102·0	105·0	103·0	102·0	102·0	102·0	100·4	+
„ 220 ..	100·2	101·6	100·4	102·0	101·8	102·6	102·4	101·2	100·4	+
„ 223 ..	101·2	101·0	101·8	102·0	101·2	101·0	101·8	103·2	100·4	—
„ 224 ..	100·4	101·6	100·6	101·2	101·0	100·8	101·4	100·8	101·0	—
„ 218 ..	101·4	101·6	101·8	101·8	102·8	103·0	103·0	102·6	101·6	—
„ 216 ..	100·2	101·0	101·0	101·8	101·2	101·4	100·6	100·4	100·2	—
„ 227 ..	101·4	103·8	103·2	104·6	104·6	102·4	102·4	102·0	101·6	—
„ 221 (control) ..	100·2	100·6	100·8	100·8	101·0	101·0	101·6	101·0	100·2	—
B. calf 9 ..	101·0	101·0	101·0	100·8	102·0	102·8	101·0	101·8	101·0	—
„ „ 3 ..	102·0	101·4	101·4	102·6	102·8	101·0	103·4	101·8	100·8	+
„ „ 1 ..	101·6	101·2	101·2	101·2	101·8	100·8	103·0	103·0	101·2	—
„ „ 2 ..	101·4	101·6	101·0	101·6	102·0	100·2	101·0	101·4	101·0	—
„ „ 6 ..	101·6	101·2	101·6	101·8	102·4	101·8	102·0	101·8	101·0	—
„ „ 5 ..	101·8	101·8	101·4	101·2	102·4	101·0	102·0	102·2	101·4	—
„ „ 13 ..	100·8	101·8	101·0	101·4	101·8	101·2	100·4	102·0	100·6	—
„ „ 4 ..	101·4	101·0	101·0	101·0	101·8	100·0	101·8	101·2	101·2	—
„ „ 11 ..	102·4	101·8	102·0	101·0	101·2	101·2	101·2	102·4	101·0	—
„ „ 8 ..	101·4	101·6	101·0	100·6	101·4	100·6	101·0	101·4	100·8	—
„ „ 10 ..	102·4	101·0	102·0	100·6	101·4	100·4	101·2	101·6	101·2	—
„ „ 12 ..	101·8	101·4	101·8	101·8	101·8	101·2	101·8	102·0	101·4	—
„ „ 7 (control) ..	99·0	100·4	101·2	100·0	100·4	100·0	101·2	101·6	100·0	—

The results of this test were very different from those obtained a month previously. Only 4 of the buffaloes (Nos. 215, 217, 222, and 220) shewed rises of temperature exceeding 2°.

A further point of difference between this test and the preceding test was the complete absence of anything in the nature of a local reaction.

It appears to be unlikely that the tuberculin was at fault as the same stock brew was used for all the tests to which these animals were subjected.

It was thought possible that the absence of temperature and local reactions was due to the fact that only one month had elapsed between the tests. The next test was therefore carried out after an interval of two months. The subcutaneous test was alone used and the dose was 3 c.c. as before.

The following table gives the temperatures recorded.

TABLE V.

Animal	9–2–20			10–2–20						Result
	10 A.M	3 P.M.	9 P.M.	9th	12th	15th	18th	21st	24th	
Buff. 215 ..	99·8	101·2	100·2	102·0	101·8	102·0	100·8	102·6	100·4	+
„ 217 ..	100·0	100·4	101·6	105·0	104·8	103·4	104·0	104·0	100·4	+
„ 219 ..	99·6	100·0	101·0	105·8	105·8	105·4	104·6	104·0	101·0	+
„ 222 ..	99·6	100·4	101·2	104·0	105·2	105·8	103·6	104·0	101·2	+
„ 220 ..	100·0	100·0	100·0	101·8	101·6	104·8	103·6	104·0	100·6	+
„ 223 ..	99·6	99·4	101·0	98·0	101·0	102·6	102·4	102·2	100·6	—
„ 224 ..	99·8	100·4	101·0	102·4	101·8	103·6	102·6	103·0	101·0	+
„ 218 ..	100·0	100·2	101·4	102·4	102·8	100·6	102·0	103·0	100·6	—
„ 216 ..	100·2	100·0	100·4	101·8	104·2	102·8	104·0	100·6	101·4	+
„ 227 ..	100·0	101·2	101·2	101·6	101·8	102·6	102·4	102·0	100·4	—
„ 221 .. (control)	99·4	101·2	100·8	100·0	100·0	101·0	98·8	100·6	100·0	—
B. calf 9 ..	100·2	101·0	101·8	103·8	103·8	104·0	106·2	103·4	102·4	+
„ „ 3 ..	99·8	101·4	100·0	100·0	101·2	101·2	101·2	100·6	101·8	—
„ „ 1 ..	100·2	101·4	101·4	102·2	102·2	102·0	102·4	102·8	100·6	—
„ „ 2 ..	99·2	99·6	100·0	101·0	101·4	102·6	100·2	100·2	101·0	+
„ „ 6 ..	100·2	101·2	101·2	101·8	102·8	102·2	103·6	103·0	102·0	+
„ „ 5 ..	100·2	101·2	100·8	101·8	101·6	103·8	103·0	103·4	102·4	+
„ „ 13 ..	99·6	101·8	103·0	102·4	102·6	104·2	102·8	102·4	101·0	—
„ „ 4 ..	99·6	100·4	101·0	101·4	102·2	102·0	103·0	102·8	100·8	+
„ „ 11 ..	100·4	101·0	101·8	102·2	102·4	102·0	102·0	102·2	100·6	—
„ „ 8 ..	99·4	101·8	102·0	102·4	102·2	103·0	102·8	102·4	101·0	—
„ „ 10 ..	101·0	100·6	101·0	101·6	102·0	103·0	103·0	103·2	102·6	+
„ „ 12 ..	100·6	100·6	100·4	101·2	101·8	103·0	102·4	102·2	100·6	+
„ „ 7 .. (control)	98·7	101·0	101·0	102·0	102·0	102·6	101·0	100·6	99·0	—

On this occasion only three of the infected buffaloes failed to react. The reactions obtained in the others were pronounced—2·4° to 4·8°.

Of the bull calves 7 reacted and 5 failed.

Neither of the controls gave any reaction.

It may be noted that in the case of calf 13 the temperature was high—103°—at the time of injection. Prior to this on the same day it had been 99·6° and 101·8° in the morning and afternoon. It is, therefore, impossible to form a definite opinion although, as judged by the standard taken, the reaction must be considered as negative.

In view of the greater proportion of positive results obtained on this occasion it appeared to be possible that it was actually an advantage to leave a period of more than one month between consecutive subcutaneous tests.

With a view, however, to testing this again and to ascertaining whether positive results could be obtained with the subcutaneous test, when it was applied at intervals of less than a month, the following plan was resorted to.

Both buffaloes and calves were divided into four groups.

As there were fewer buffaloes than calves the groups of the former comprised 2, 2, 3 and 3, while the calves were divided into four equal groups of three each.

A group of buffaloes and a group of calves were tested along with the controls at intervals of a week, a fortnight, three weeks, and a month after the test of February 9th.

In selecting the animals for these tests, those which had reacted with the greatest constancy were placed as far as possible in the earlier groups, the inference being that as they had reacted before they would be more likely to react again, and that therefore they would serve better as a test for determination of the intervals at which two tests could be applied than those which had been uncertain in reacting previously.

The following table gives the results of the test carried out on February 16th, 1920:—

TABLE VI.

Animal	16-2-20			17-2-20						Result
	10 A.M.	3 P.M.	9 P.M.	9th	12th	15th	18th	21st	24th	
Buff. 216 ..	100·0	100·0	100·2	103·0	102·0	104·4	101·6	101·8	100·6	+
„ 217 ..	100·0	100·2	102·0	103·4	105·2	103·4	102·2	100·4	101·2	+
„ 221 .. (control)	100·6	101·2	101·2	100·4	99·6	100·4	101·4	99·2	100·0	—
B. calf 2 ..	100·6	100·0	99·6	101·6	103·2	103·2	102·0	101·4	104·0	+
„ „ 4 ..	100·8	100·0	100·4	102·0	102·6	103·2	102·6	101·8	100·6	+
„ „ 5 ..	101·2	100·0	101·4	103·0	104·2	103·2	103·6	101·8	100·4	+
„ „ 7 .. (control)	99·3	100·6	101·8	100·0	100·0	100·2	100·2	101·0	100·4	—

From this it will be seen that all the infected animals reacted; the rises ranged from 2·8° to 4·2°. Neither of the controls reacted.

Table VII shows the reactions obtained in the buffaloes and calves which were subjected to the subcutaneous test a fortnight after their previous test.

TABLE VII.

Animal	23-2-20			24-2-20						Result
	10 A.M.	3 P.M	9 P.M.	9th	12th	15th	18th	21st	24th	
Buff. 219 ..	99·6	101·6	102·4	104·6	105·0	102·5	105·0	102·4	101·0	+
,, 220 ..	100·4	101·8	101·6	102·0	103·8	103·2	102·6	102·0	101·4	+
,, 221 (control) ..	100·8	100·4	101·8	101·0	101·8	102·0	103·0	102·0	100·4	—
B. calf 6 ..	101·4	102·2	101·8	101·4	101·8	101·0	102·6	101·4	101·4	
,, ,, 8 ..	101·0	101·8	101·6	102·4	103·6	103·2	105·0	105·0	102·4	+
,, ,, 9 ..	101·2	101·8	101·2	101·4	102·0	103·6	105·2	103·8	102·0	+
,, ,, 7 (control) ..	100·6	101·8	100·2	100·0	101·2	101·2	101·0	101·0	100·8	—

On this occasion both the inoculated buffaloes reacted 3·2° and 4·2°, while the control did not.

Of the bull calves, one, No. 6, failed to react, the rise being only 0·8°. This animal had reacted a fortnight before. The other infected animals gave reactions of 3°, 4° and 4°, respectively. The bull calf control did not react.

Table VIII gives the results of the tuberculin test of the group of animals tested at an interval of three weeks after the previous test.

TABLE VIII.

Animal	1-3-20			2-3-20						Result
	10 A.M.	3 P.M.	9 P.M.	9th	12th	15th	18th	21st	24th	
Buff. 224 ..	100·2	101·4	101·0	103·0	103·0	103·0	102·0	101·2	101·4	+
,, 223 ..	99·8	102·6	102·0	103·2	103·0	103·0	102·6	102·0	101·0	—
,, 222 ..	100·5	101·6	101·8	104·4	104·4	104·0	104·0	101·4	101·2	+
,, 221 (control) ..	100·0	100·0	101·4	100·0	100·2	100·4	100·0	100·0	99·8	—
B. calf 12 ..	101·5	101·8	102·0	103·2	102·8	102·4	102·4	102·4	101·2	—
,, ,, 10 ..	101·0	102·0	101·8	102·4	102·4	103·0	102·6	101·4	101·8	—
,, ,, 11 ..	100·4	103·4	101·8	103·0	101·6	100·6	102·4	103·0	101·4	—
,, ,, 7 (control) ..	100·4	100·6	101·8	100·0	100·0	100·0	101·0	101·6	101·0	—

On this occasion only two animals—Buffaloes 224 and 222—gave positive reactions.

Tuberculin test carried out with the last group of animals was one month after the previous test (Table IX).

TABLE IX.

Animal	8-3-20			9-3-20						Result
	10 A.M.	3 P.M.	9 P.M.	9th	12th	15th	18th	21st	24th	
Buff. 215 ..	100·2	100·4	99·8	101·2	102·4	100·2	100·0	102·4	100·2	+
„ 218 ..	100·2	100·6	101·0	101·8	100·2	99·4	100·0	102·2	100·0	—
„ 227 ..	100·2	100·4	100·0	102·0	101·8	101·2	100·0	101·2	100·4	+
„ 221 (control) ..	100·4	100·6	100·8	100·8	101·0	100·4	102·0	101·4	100·4	—
B. calf 1 ..	100·4	100·6	101·0	100·4	101·0	101·4	101·0	100·0	100·2	—
„ „ 3 ..	100·4	100·2	101·0	100·4	101·0	101·4	100·4	101·2	100·4	—
„ „ 13 ..	100·4	100·6	100·4	102·0	101·0	101·4	101·0	100·0	100·2	—
„ „ 7 (control) ..	101·8	100·4	101·4	100·0	101·0	101·6	102·0	101·0	100·0	—

On the occasion of this test only two buffaloes reacted, No. 215, 2·6° and No. 227, 2°.

One feels a little diffidence in accepting either of these tests as positive in spite of the fact that a rise of 2° or more was registered. In the case of No. 215 this arises from the fact that the temperature was very low, 99·8° at the time of injection, and from the fact that the temperature did not rise and fall in a steady manner. The maximum of 102·4° was recorded at the 12th and 21st hours, but the temperature fell to 100·2° and 100° at the two intervening periods.

In the case of No. 227, the rise was exactly 2° and the maximum was registered at the 9th hour. It is true that after this the temperature fell in a regular manner up to the 18th hour. This may possibly have been a case in which the reaction occurred early. Had the temperatures been taken at the 3rd and 6th hours, a regular curve might have been established.

In all, 88 subcutaneous tests have been carried out upon animals inoculated with culture of tubercle bacilli, and the following table shows a summary of the results ;—

TABLE X.

Animal	10–11–19	11–12–19	9–2–20	16–2–20	23–2–20	1–3–20	8–3–20
Buff. 215 ..	+	+	+	..	..	..	+
„ 217 ..	+	+	+	+	..	..	..
„ 219 ..	+	−	+	..	+	..	..
„ 222 ..	+	+	+	..	..	+	..
„ 220 ..	+	+	+	..	+	..	..
„ 223 ..	+	−	−	..	..	−	..
„ 224 ..	+	−	+	..	..	+	..
„ 218 ..	+	−	−	..	..	..	−
„ 216 ..	+	−	+	+	..	..	..
„ 227 ..	+	−	−	..	..	..	+
„ 221 .. (control)	−	−	−	−	−	−	−
B. calf 9 ..	−	−	+	..	+	..	..
„ „ 3 ..	−	+	−	..	..	..	−
„ „ 1 ..	+	−	−	..	..	..	−
„ „ 2 ..	+	−	+	+	..	..	..
„ „ 6 ..	+	−	+	..	−	..	..
„ „ 5 ..	+	−	+	+	..	..	..
„ „ 13 ..	+	−	−	..	..	..	−
„ „ 4 ..	+	−	+	+	..	..	..
„ „ 11 ..	−	−	−	..	..	−	..
„ „ 8 ..	+	−	−	..	+	..	..
„ „ 10 ..	+	−	+	..	..	−	..
„ „ 12 ..	−	−	+	..	..	−	..
„ „ 7 .. (control)	−	−	−	−	−	−	−

Applying the standard already decided upon, that is to say, a rise of 2° or more above the temperature at the time of injection to constitute a positive reaction, it is found that positive results were obtained in 50 instances, *i.e.*, roughly 57 per cent.

If the buffaloes and calves be considered separately it is found that a positive result was obtained in 29 out of 40 tests, or 72·5 per cent., in the case of the buffaloes, and in 21 out of 48, or about 44 per cent., in the case of calves.

The buffaloes gave, therefore, a far higher percentage of accurate results than the plains calves.

The following reactions require special mention: They are all included, as judged strictly by the standard, among the negative results.

Buffalo 227. Test of December 11th, 1919. This animal's temperature was 103·2° at the time of injection and rose to 104·6° at the 9th and 12th hours, subsequently steadily falling to 101·6° at the 24th hour. It must be noted, however, that the temperatures taken at 10 A.M. and 3 P.M. on the day of the test were 101·4° and 103·8°, respectively. It is obvious that there was a rise of an accidental nature just at the time when the tuberculin was injected.

The whole of the animals used had had their temperatures taken twice daily for six weeks prior to the start of the experiment. Reference to the

charts of buffalo 227 shows that, as is very frequently the case with plains buffaloes brought to the hills, especially during the cold weather, the temperature was very variable. During the six weeks prior to the experiment a temperature of 103° or more was registered on 14 days. These were almost invariably evening temperatures.

After the animal had been infected sudden rises to nearly 106° were recorded on three or four occasions. Examination of the blood on these occasions always gave a negative result, and the temperature fell to normal within 48 hours.

This animal quite clearly failed to react when tested two months later, but it just attained to the standard of a positive result when tested on March 8th, 1920.

Buffalo 223. Test of February 9th, 1920. The accuracy of the result in this case, is brought into question because, although the rise of temperature was only 1·6° (101°—102·6°) as judged by the standard, the animal's temperature had been 99·6° and 99·4° at 10 A.M. and 3 P.M. on the day of the test, and was 98° at the 9th hour. This animal had had a very unsteady temperature during the six weeks prior to the test, and both trypanosomes and piroplasms had been detected in its blood. It is remarkable, however, that the animal gave a definitely positive reaction to the first test only (Nov. 10, 1919).

Bull calf 13. Test of February 9th, 1920. In the case of this animal the temperature was high at the time of injection—103°. In the morning and afternoon of the same day it had been 99·6° and 101·8°. It would therefore seem that a rise was acutally in progress at the time of injection, just as in the case of buffalo 227. The maximum temperature was 104·2° at the 15th hour, the temperature curve was very regular, and had the initial temperature been normal, the reaction would have been definitely positive.

Examination of this animal's charts shows that during the six weeks prior to the experiment the evening temperature had been 103° or more for sometimes nine or ten days in succession, the morning temperature being about 102°. This calf, like buffalo 223, gave a definitely positive reaction to the first test only.

One of the most remarkable points about the whole series of tests is the extraordinary number of failures to react recorded on December 11th. Only 4 buffaloes gave definitely positive reaction on this occasion. That this was not due to the shortness of the interval between this test and the preceding one—one month—is indicated by the fact that two buffaloes and three

calves, which had reacted on February 9th, reacted again on February 16th.

Bull calf 11 failed to react to every test.

The number of tests carried out is too small to base any definite conclusions upon. The fact that all the animals used were brought from the plains and the cooler climate may have affected the results obtained. The results would, however, appear to indicate that the subcutaneous test carried out under the conditions of these experiments is nothing like so reliable as it is in Europe.

Results of infection.

In Glen Liston's experiments, to which reference has been made, two buffaloes died of acute, generalised tuberculosis, five when killed shewed retrogressive lesions, two died of broncho-pneumonia soon after inoculation, and two of causes other than tuberculosis. Of the calves, six died of acute, generalised tuberculosis, and six when killed shewed retrogressive lesions.

None of the animals used were allowed to survive beyond the 142nd day. All but two had died or had been killed prior to this date.

In the experiments here recorded none of the animals inoculated died. None of them had shewn any evidence of ill-health during the course of the experiment, and when killed all were in good condition, some were in fact quite fat.

With a view to rendering the experiment comparable to that carried out by Glen Liston, it was decided to kill off all the animals as quickly as possible after the completion of the 5th month.

A start was accordingly made on March 11th—152 days after the inoculations—and the whole had been destroyed by the 18th.

In making the post-mortem examinations the following organs and glands were examined: Lungs, liver, spleen, kidneys, prescapular, prepectoral, sternal, precrural, bronchial, mediastinal, portal, mesenteric and pharyngeal glands, and the lesion at the seat of inoculation.

The lungs were first examined intact and were then cut into slices about 2 inches in thickness. Each slice was passed between the fingers. The other organs were treated in the same way save that the cuts made into the more solid organs, liver and kidneys, were closer together, not more than $\frac{1}{4}$ to $\frac{1}{2}$ inch apart. It is possible that minute lesions in these may have escaped detection, but it is certain that none were overlooked in the lungs and spleen. All

the glands referred to were carefully cut into thin slices for examination, unless their condition was obvious without cutting them more than once. In the post-mortem notes only those organs and glands in which lesions were found are mentioned, but all those referred to above were examined in every case.

Individual histories of each animal.

(*Where tuberculin tests are mentioned only the subcutaneous tests are referred to.*)

Buffalo calf 215. Age 1 year. Weight 210 lb.

8–10–19. Tuberculin test. Negative.

11–10–19. Inoculated subcutaneously with 10 mg. of culture.

10–11–19. Tuberculin test. Positive.

11–12–19. Do. do. do.

9– 2–20. Do. do. do.

8– 3–20. Do. do. do.

12– 3–20. Killed. Weight 305 lb.

At the seat of inoculation a rounded mass of dry, nearly white, necrotic tissue, which was gritty to the touch and measured about an inch in diameter.

In the upper part of the left prescapular gland an agglomeration of some thirty calcified pin-head tubercles.

Buffalo 217. Age 1 year. Weight 255 lb.

8–10–19. Tuberculin test. Negative.

11–10–19. Inoculated subcutaneously with 10 mg. of culture.

10–11–19. Tuberculin test. Positive.

11–12–19. Do. do. do.

9– 2–20. Do. do. do.

16– 2–20. Do. do. do.

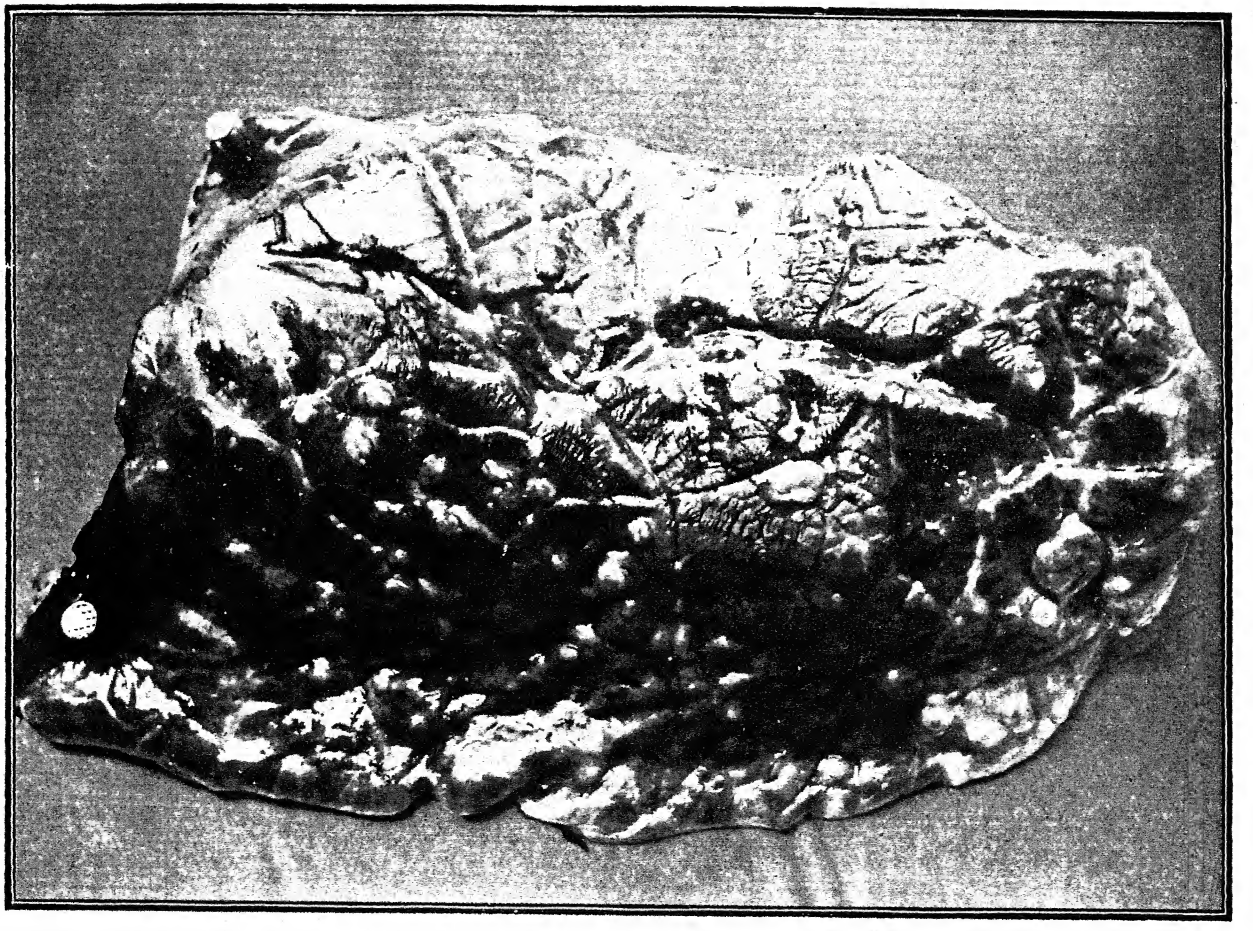
On January 19th an abscess was opened at the seat of inoculation and a small quantity of pus evacuated.

12–3–20. Killed. Weight 370 lb.

At the seat of inoculation the subcutaneous tissue contained a group of calcified tubercles as large as millet seeds.

The left prescapular gland contained five dry, necrotic centres which were of a very pale yellow colour and partly calcified. They ranged in size from a pea to a small nut.

Each of the bronchial and mediastinal glands contained 3 or 4 partly calcified tubercles of the size of millet seeds.



Anterior lobe of the right lung of Buffalo 219, shewing the tuberculous pleurisy. (About natural size.)

The spleen contained a single lesion of the same kind. Microscopic examination of this lesion shewed that it was encapsuled.

Buffalo 219. Age 1 year. Weight 260 lb.

8–10–19. Tuberculin test. Negative.

11–10–19. Inoculated subcutaneously with 10 mg. of culture.

10–11–19. Tuberculin test. Positive.

11–12–19. Do. do. Negative.

9– 2–20. Do. do. Positive.

23– 2–20. Do. do. do.

On January 19th an abscess was opened at the seat of inoculation and a small quantity of pus evacuated.

12–3–20. Killed. Weight 330 lb.

At the seat of inoculation there was an encapsuled collection of gritty caseo-purulent material measuring about 2 inches by one. In the connective tissue surrounding this there were about a score of calcified tubercles as large as peas.

The prescapular gland was twice the normal size and its substance was almost entirely converted into nearly white, firm, dry, necrotic tissue in which were embedded gritty particles.

The lungs were closely beset with partly calcified tubercles varying in size from a millet seed to a bean. There was no evidence of softening in the larger lesions.

The left kidney contained a single lesion about the size of a lentil shewing a thick fibrous capsule and a speck of pale yellow, dry, necrotic tissue in the centre. The right kidney contained five such lesions.

The spleen contained five millet seed lesions.

The pleura over the anterior halves of both lungs shewed a considerable number of small, pale yellow, flat projections, varying in size from a split pea downwards. (Plate II.)

The bronchial and mediastinal glands were all about three times the normal size and their tissue was almost entirely pale yellow in colour, firm, dry and necrotic.

The hepatic glands contained six tubercles as large as peas; a lesion of doubtful nature was found in the substance of the liver.

Three millet seed lesions were found in the mesenteric glands.

The left prepectoral and sternal glands all contained millet see tubercles.

Buffalo 222. Age 1 year. Weight 300 lb.

8–10–19. Tuberculin test. Negative.

11–10–19. Inoculated subcutaneously with 10 mg. of culture.
10–11–19. Tuberculin test. Positive.
11–12–19. Do. do. do.
9–2–20. Do. do. do.
1–3–20. Do. do. do.
11–3–20. Killed. Weight 410 lb.

At the seat of inoculation on the left side of the neck there was a pale yellow, dry, necrotic piece of tissue about as large as a horse bean which was gritty at points.

The left prescapular gland was about four times the normal size, and the whole of its outer part was converted into a dry, pale yellow, necrotic tissue which was gritty to the knife. (Plate III.)

About half the substance of the gland was necrotic.

Both right and left bronchial glands contained small, dry, tuberculous centres about as large as peas which were partly calcified.

Both anterior and posterior mediastinal glands presented the same appearance.

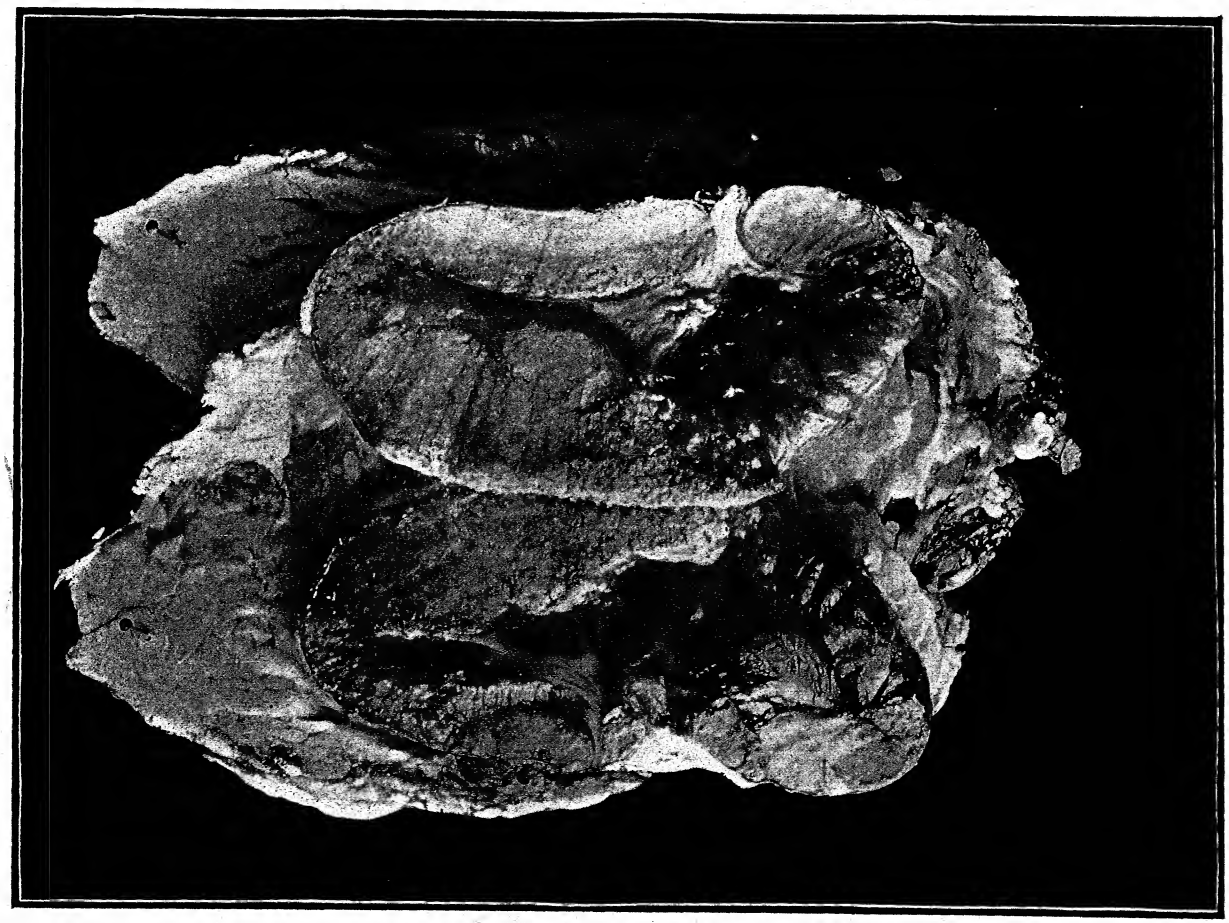
Buffalo 220. Age 1 year. Weight 330 lb.
8–10–19. Tuberculin test. Negative.
11–10–19. Inoculated subcutaneously with 10 mg. of culture.
10–11–19. Tuberculin test. Positive.
11–12–19. Do. do. do.
9–2–20. Do. do. do.
23–2–20. Do. do. do.
11–3–20. Killed. Weight 492 lb.

The local lesion took the form of a quantity of pale greenish pus measuring about an ounce.

The left prescapular gland contained a score of dry-looking, opaque tubercles rather smaller than peas.

Buffalo 223. Age 1 year. Weight 225 lb.
8–10–19. Tuberculin test. Negative.
11–10–19. Inoculated subcutaneously with 50 mg. of culture.
10–11–19. Tuberculin test. Positive.
11–12–19. Do. do. Negative.
9–2–20. Do. do. do.
1–3–20. Do. do. do.
11–3–20. Killed. Weight 288 lb.

At the seat of inoculation there was an area of firm, dry, pale yellow, necrotic tissue about the size and shape of a preserved fig.



Left prescapular gland of Buffalo 222 almost entirely converted into firm dry tuberculous tissue.
(A little smaller than natural size.)
The quantity of fat around the gland is an index of the good condition of the animal at the time of slaughter.

The prescapular gland on the same side shewed a narrow margin of pale yellow, necrotic tissue containing gritty particles.

The left pharyngeal contained two small tuberculous centres, and the right one each about as large as lentils.

The lungs contained large numbers of tubercles, a little larger than pins' heads. It was estimated that the nodules numbered about 20 per cubic inch of lung tissue.

The spleen contained some fifty tubercles about as large as millet seeds.

Microscopic examination of the splenic lesions shewed that a fibrous capsule was in the process of formation around them.

Buffalo 224. Age 1 year. Weight 255 lb.

8–10–19. Tuberculin test. Negative.
11–10–19. Inoculated subcutaneously with 50 mg. of culture.
10–11–19. Tuberculin test. Positive.
11–12–19. Do. do. Negative.
9–2–20. Do. do. Positive.
1–3–20. Do. do. do.
11–3–20. Killed. Weight 415 lb.

The local lesion took the form of about two ounces of thick pus of a slightly greenish tint enclosed in a thick, fibrous capsule.

The prescapular gland on the same side contained four tuberculous centres ranging in size from a pea to a hazel nut, and composed of nearly white necrotic tissue.

Buffalo 218. Age 1 year. Weight 270 lb.

8–10–19. Tuberculin test. Negative.
11–10–19. Inoculated subcutaneously with 50 mg. of culture.
10–11–19. Tuberculin test. Positive.
11–12–19. Do. do. Negative.
9–2–20. Do. do. do.
8–3–20. Do. do. do.
12–3–20. Killed. Weight 370 lb.

The local lesion took the form of a dry, pale yellow, firm piece of necrotic tissue of the size and shape of a preserved fig.

The left prescapular gland was about six times the normal size and the normal tissue was almost entirely replaced by dry, firm, pale yellow, necrotic tissue which was gritty to the touch.

The left precrual gland contained three necrotic centres as large as millet seeds.

The peritoneum covering the liver, spleen, stomachs and omentum was more or less covered with delicate, feathery outgrowths. In a few places the peritoneum shewed little, pinkish grey, flat outgrowths measuring one-eighth to one-fourth of an inch in diameter.

The spleen contained about 50 millet seed lesions.

The hepatic glands were crowded with lesions a little larger than pins' heads.

The lungs, and more particularly the posterior halves of the main lobes, were crowded with tubercles varying in size up to that of a pea. The apical lobes had practically escaped infection. The majority of the lesions were firm and dry, but in some of the larger ones there was a certain amount of softening.

From the naked eye appearance, the lesions seemed to be enclosed in thin, fibrous capsules.

Buffalo 216. Age 1 year. Weight 280 lb.

8–10–19. Tuberculin test. Negative.
11–10–19. Inoculated subcutaneously with 50 mg. of culture.
10–11–19. Tuberculin test. Positive.
11–12–19. Do. do. Negative.
9–2–20. Do. do. Positive.
16–2–20. Do. do. do.
12–3–20. Killed. Weight 420 lb.

At the seat of inoculation there was an irregularly shaped mass of dry, pale yellow, necrotic tissue measuring about 2 inches in each direction.

The prescapular gland shewed no visible lesions. This gland was sliced most carefully into layers not more than one-eighth of an inch thick and each was carefully scrutinised, but no lesions were detected.

The right lung contained a single nodular lesion of doubtful nature.

Buffalo 227. Age 1 year. Weight 315 lb.

8–10–19. Tuberculin test. Negative.
11–10–19. Inoculated subcutaneously with 50 mg. of culture.
10–11–19. Tuberculin test. Positive.
11–12–19. Do. do. Negative.
9–2–20. Do. do. do
9–3–20. Do. do. Positive.
15–3–20. Killed. Weight 460 lb.

The local lesion took the form of an encapsuled mass of caseo-purulent material which was gritty to the touch, and was about the size of two fingers.

One-third of the left prescapular gland was converted into dry, firm, white necrotic tissue.

The right bronchial gland contained four tubercles rather larger than pins' heads.

The parietal and visceral pleuræ shewed a fine, greyish pink, translucent, granular growth over the greater part of their extent. There was also a pinkish, fringe-like growth along the lower border of the main lobe of both lungs.

The greater part of both visceral and parietal peritoneum shewed a granular growth similar to that found in the chest cavity.

The left prepectoral gland shewed four or five tubercles as large as split peas.

Buffalo 221. Age 1 year. Weight 238 lb.

8–10–19. Tuberculin test. Negative.
11–10–19. Control. Not inoculated with culture.
11–12–19. Tuberculin test. Negative.
9–2–20. Do. do. do.
16–2–20. Do. do. do.
23–2–20. Do. do. do.
1–3–20. Do. do. do.
8–3–20. Do. do. do.
15–3–20. Killed. Weight 400 lb.

No evidence of tuberculosis.

Bull calf 9. Age 10 months. Weight 140 lb.

8–10–19. Tuberculin test. Negative.
11–10–19. Inoculated subcutaneously with 10 mg. of culture.
10–11–19. Tuberculin test. Negative.
11–12–19. Do. do. do.
9–2–20. Do. do. Positive.
23–2–20. Do. do. do.
16–3–20. Killed. Weight 225 lb.

The local lesion took the form of an encapsuled abscess as large as a walnut containing caseo-purulent material.

The left prescapular gland contained about a dozen caseo-calcareous tubercles at each end.

Each of the bronchial glands contained about half a dozen millet seed lesions.

The right lung contained a single caseo-calcareous lesion as large as a millet seed.

There were slight traces of both visceral and parietal tuberculous pleurisy.

The mediastinal glands contained four caseous pin-head lesions.

There was extensive visceral and parietal tuberculous peritonitis, the lesion being in the early stage, *i.e.*, a thin layer of pinkish grey, granular tissue.

Bull calf 3. Age 8 months. Weight 152 lb.

8–10–19. Tuberculin test. Negative.
11–10–19. Inoculated subcutaneously with 10 mg. of culture.
10–11–19. Tuberculin test. Negative.
11–12–10. Do. do. Positive.
9–2–20. Do. do. Negative.
8–3–20. Do. do. do.
18–3–20. Killed. Weight 260 lb.

At the seat of inoculation an encapsuled collection of yellow gritty caseo-purulent material as large as a date.

The left prescapular gland contained three caseo-calcareous centres as large as peas, the left bronchial contained two, and the mediastinal glands three pin-head calcareous tubercles. A single similar lesion was present in one of the portal glands.

Bull calf 1. Age 1 year. Weight 170 lb.

8–10–19. Tuberculin test. Negative.
11–10–19. Inoculated subcutaneously with 10 mg. of culture.
10–11–19. Tuberculin test. Positive.
11–12–19. Do. do. Negative.
9–2–20. Do. do. do.
8–3–20. Do. do. do.
16–3–20. Killed. Weight 235 lb.

At the seat of inoculation an encapsuled abscess as large as a walnut containing soft caseo-purulent material which was gritty to the touch.

The left prescapular gland contained a dozen caseo-purulent centres about as large as peas.

The spleen contained a score of centres of the same size. These appeared to be composed of a number of smaller, translucent, pin-head centres. Some minute specks of caseous material were visible. Microscopic examination shewed that these nodules contained a distinct net work of connective tissue both between and within the component tubercles.

The liver contained one encapsuled millet seed lesion.

The posterior mediastinal gland contained a single lesion as large as a millet seed.

The bronchial glands were enlarged, but there were no definite tubercles visible.

Bull calf 2. Age 1 year. Weight 235 lb.
8–10–19. Tuberculin test. Negative.
11–10–19. Inoculated subcutaneously with 10 mg. of culture.
10–11–19. Tuberculin test. Positive.
11–12–19. Do. do. Negative.
9–2–20. Do. do. Positive.
16–2–20. Do. do. do.

Abscess at the seat of inoculation opened on December 9th. About one ounce of pus was evacuated.

16–3–20. Killed. Weight 370 lb.

The local lesion took the form of an encapsuled abscess as large as a marble containing yellow, gritty pus.

The left prescapular gland contained three caseo-calcareous lesions as large as peas.

The posterior mediastinal gland contained a single caseous lesion as large as a millet seed.

The lower border of both lungs shewed a small amount of tuberculous fringe-like growth.

Bull calf 6. Age 1 year. Weight 245 lb.
8–10–19. Tuberculin test. Negative.
11–10–19. Inoculated subcutaneously with 10 mg. of culture.
10–11–19. Tuberculin test. Positive.
11–12–19. Do. do. Negative.
9–2–20. Do. do. Positive.
23–2–20. Do. do. Negative.
15–3–20. Killed. Weight 400 lb.

Careful search failed to reveal any evidence of a lesion at the seat of inoculation.

The left prescapular gland contained half a dozen minute yellow, gritty particles.

Both lungs were closely peppered with very small ecchymoses, but there were no appreciable nodules.

Bull calf 5. Age 1 year. Weight 275 lb.
8–10–19. Tuberculin test. Negative.
11–10–19. Inoculated subcutaneously with 10 mg. of culture.
10–11–19. Tuberculin test. Positive.
11–12–19. Do. do. Negative.
9–2–20. Do. do. Positive.
16–2–20. Do. do. do.

18–3–20. Killed. Weight 450 lb.

At the seat of inoculation there was an encapsuled collection of gritty caseo-pus about 2 inches by one in size.

The left prescapular gland contained about a dozen caseo-calcareous centres as large as millet seeds.

Bull calf 13. Age 1 year. Weight 140 lb.

8–10–19. Tuberculin test. Negative.
11–10–19. Inoculated subcutaneously with 50 mg. of culture.
10–11–19. Tuberculin test. Positive.
11–12–19. Do. do. Negative.
9–2–20. Do. do. do.
8–3–20. Do. do. do.

Abscess at the seat of inoculation opened on November 21st. About two ounces of thick pus removed.

18–3–20. Killed. Weight 180 lb.

At the seat of inoculation an encapsuled collection of yellow caseo-pus as large as a walnut.

The left prescapular gland contained four caseo-calcareous tubercles as large as peas.

Bull calf 4. Age 1 year. Weight 160 lb.

8–10–19. Tuberculin test. Negative.
11–10–19. Inoculated subcutaneously with 50 mg. of culture.
10–11–19. Tuberculin test. Positive.
11–12–19. Do. do. Negative.
9–2–20. Do. do. Positive.
16–2–20. Do. do. do.
18–3–20. Killed. Weight 255 lb.

At the seat of inoculation a nodule of dense, brownish, fibrous tissue as large as a filbert nut with a caseo-calcareous centre as large as a millet seed.

The left prescapular gland was cut into slices less than one eighth of an inch in thickness and these were searched thoroughly, but no visible lesions could be found.

Bull calf 11. Age 1 year. Weight 200 lb.

8–10–19. Tuberculin test. Negative.
11–10–19. Inoculated subcutaneously with 50 mg. of culture.
10–11–19. Tuberculin test. Negative.
11–12–19. Do. do. do.
9–2–20. Do. do. do.

1–3–20. Tuberculin test. Negative.

Abscess opened at the seat of inoculation on November 21st. About one and a half ounces of thick pus removed.

16–3–20. Killed. Weight 310 lb.

At the seat of inoculation there were eight encapsuled abscesses as large as marbles containing caseo-purulent material.

The left prescapular gland contained five centres of soft, yellow, caseous material ranging in size from a pea to a walnut.

The spleen contained five encapsuled centres of soft, caseous material as large as peas.

The liver contained a single small encapsuled and calcified lesion.

The lungs were somewhat closely beset with encapsuled caseous lesions rather smaller than peas. It was estimated that these lesions numbered about six per cubic inch of lung tissue.

The bronchial and mediastinal glands were all some four times the normal size and were packed with caseous lesions.

The parietal pleura shewed the earliest stage of tuberculous pleurisy over a few small areas.

The left prepectoral glands were about twice the normal size and entirely converted into soft yellow caseous material.

Bull calf 8. Age 1 year. Weight 220 lb.

8–10–19. Tuberculin test. Negative.

11–10–19. Inoculated subcutaneously with 50 mg. of culture.

10–11–19. Tuberculin test. Positive.

11–12–19. Do. do. Negative.

9–2–20. Do. do. do.

23–2–20. Do. do. Positive.

Abscess at the seat of inoculation opened on November 21st. About an ounce of thick pus obtained.

16–3–20. Killed. Weight 295 lb.

Two encapsuled abscesses as large as marbles marked the seat of inoculation.

The left prescapular gland contained a single gritty lesion as large as a millet seed.

The parietal pleura shewed diffuse tuberculous pleurisy. The greater part of it was covered with a thin layer of greyish pink, granular tissue.

The visceral pleura shewed similar lesions but to a less extent. In one or two places distinct grape-like projections were in the process of formation. The largest of these was about the size of a horse bean.

Bull calf 10. Age 1 year. Weight 245 lb.

8–10–19. Tuberculin test. Negative.
11–10–19. Inoculated subcutaneously with 50 mg. of culture.
10–11–19. Tuberculin test. Positive.
11–12–19. Do. do. Negative.
9–2–20. Do. do. Positive.
1–3–20. Do. do. Negative.
15–3–20. Killed. Weight 360 lb.

At the seat of inoculation there was an encapsuled abscess containing about two ounces of thick, gritty, yellow pus.

The left prescapular gland was three times the normal size and contained a centre of dry, yellow, caseous material as large as a marble.

The left bronchial gland contained four yellow pin-head lesions.

The portal glands shewed three similar lesions.

In the substance of the liver there were about a dozen minute, opaque, yellow centres.

Bull calf 12. Age 1 year. Weight 310 lb.

8–10–19. Tuberculin test. Negative.
11–10–19. Inoculated subcutaneously with 50 mg. of culture.
10–11–19. Tuberculin test. Negative.
11–12–19. Do. do. do.
9–2–20. Do. do. Positive.
1–3–20. Do. do. Negative.

Abscess opened at the seat of inoculation on November 9th. About one ounce of thick pus removed.

15–3–20. Killed. Weight 455 lb.

An encapsuled collection of soft, yellow, caseous material as large as a marble marked the seat of inoculation.

The left prescapular gland was about eight times the normal size and contained nine yellow caseous centres, varying in size from a pea to a walnut.

The left prepectoral gland was twice the normal size and entirely converted into yellow, caseous material.

The bronchial and mediastinal glands were closely beset with yellow caseous centres as large as peas.

The lungs contained a number of small shot-like tubercles which appeared to be encapsuled (two or three per cubic inch of lung tissue).

The spleen contained three necrotic centres as large as lentils.

The liver contained four millet seed lesions.

Bull calf 7. Age 1 year. Weight 360 lb.

8–10–19. Tuberculin test. Negative.

Control. Not inoculated with culture.

10–11–19. Tuberculin test. Negative.

11–12–19. Do. do. do.

9–2–20. Do. do. do.

16–2–20. Do. do. do.

23–2–20. Do. do. do.

1–3–20. Do. do. do.

8–3–20. Do. do. do.

18–3–20. Killed. Weight 495 lb.

No evidence of tuberculosis.

The post-mortem findings have been classified as (*a*) slight; (*b*) extensive but not severe; (*c*) generalised, but not progressive.

The significance of these definitions is as follows:—

(*a*) Slight tuberculosis. A lesion at the seat of inoculation. Lesions in the thoracic glands and possibly the abdominal glands. A few lesions in the lungs or spleen.

(*b*) Extensive, but not severe. As the above but with the addition of tuberculous pleurisy or peritonitis or both. No cases of extensive tuberculosis of a severe nature were found.

(*c*) Generalised, but not progressive. Cases in which there was evidence of invasion of the organs by way of the blood stream, with the production of numerous lesions in the lungs.

It is to be noted that, although there was evidence of generalisation in some of the animals, in no case could it be considered as an acute, generalised infection. The survival of the animals for over five months after infection, during which time they gained very considerably in weight and failed to show any clinical symptoms, the good condition of the carcases at the time of slaughter, and the encapsulation of the lesions found, all indicate that, although there was a distribution of the bacilli by the blood, the disease thus produced was not of a progressive character.

From the Report of the Royal Commission on Tuberculosis it may be gathered (*Second Interim Report*, Part II, Appendix, Vol. I, p. 34) that in the experiments carried out by the Commission the subcutaneous injection of 50 mg. almost invariably produced fatal, generalised tuberculosis within seven weeks. When the dose was 10 mg. a similar result was produced in 60 per cent. of cases within 8 weeks.

TABLE XI.

Animal	Dose	Gain in weight	Duration of life	Post-mortem findings
Buff. 215	10 mg.	95 lb.	153 days	Slight tuberculosis.
„ 217	„	115 „	„ „	„ „
„ 219	„	70 „	„ „	Generalised, but not progressive.
„ 222	„	110 „	152 „	Slight tuberculosis.
„ 220	„	162 „	„ „	„ „
„ 223	50 „	63 „	„ „	Generalised, but not progressive.
„ 224	„	160 „	„ „	Slight tuberculosis.
„ 218	„	100 „	153 „	Generalised, but not progressive.
„ 216	„	140 „	„ „	Slight tuberculosis.
„ 227	„	145 „	156 „	Extensive, but not severe.
„ 221	Cont.	162 „	„ „	Healthy.
B. calf 9	10 mg.	85 „	157 „	Extensive, but not severe.
„ „ 3	„	108 „	159 „	Slight tuberculosis.
„ „ 1	„	65 „	157 „	„ „
„ „ 2	„	135 „	„ „	„ „
„ „ 6	„	155 „	156 „	„ „
„ „ 5	„	175 „	159 „	„ „
„ „ 13	50 „	40 „	„ „	„ „
„ „ 4	„	95 „	„ „	„ „
„ „ 11	„	110 „	157 „	Generalised, but not progressive.
„ „ 8	„	75 „	„ „	Extensive, but not severe.
„ „ 10	„	115 „	156 „	Slight tuberculosis.
„ „ 12	„	145 „	„ „	Generalised, but not progressive.
„ „ 7	Cont.	135 „	159 „	Healthy.

It is seen that of the buffaloes six shewed slight tuberculosis, one shewed extensive but not severe infection, and three shewed generalised but not progressive disease. Four out of the five to which 10 mg. of culture were given shewed slight disease, the remaining one shewing generalised disease.

Of the calves eight shewed slight tuberculosis, two extensive and two generalised infection. Of the six inoculated with 10 mg. five shewed slight infection and one extensive disease.

In both species of animals, therefore, the larger dose produced somewhat more severe effects than the smaller dose. It is not possible to form any definite opinion as to whether there is any difference in susceptibility between the buffaloes and the bull calves, but the lesions found in the buffaloes affected with generalised tuberculosis were somewhat more marked than in the calves similarly affected.

Assuming that the susceptibility of plains buffaloes and cattle in the United Provinces is similar to that possessed by animals in the Bombay Presidency, a comparison of the results here recorded with those obtained by Glen Liston indicates that Indian strains of tubercle bacilli isolated from cattle are far less virulent than European bovine strains.

For convenience the tabular statement of the results of Glen Liston's experiments is reproduced. (Table XII.) The arrangement of the columns has been changed to conform with the arrangement of Table XI.

It might be objected that the particular strain used for these cattle experiments was of particularly low virulence (Ferozepore No. 118). That this was in all probability not the case is shewn by the experiments carried out with other strains on guinea-pigs.

It is not suggested that definite conclusions as to the comparative virulence of the various strains used can be drawn from a comparison of the duration of life of the guinea-pigs inoculated. The very fact that the materials used for the inoculations were derived from natural lesions and that no estimate of their bacterial content was made is sufficient to render such a comparison impossible. In addition to this, as the purpose of the inoculations was to permit of the isolation of the various strains, at the most four animals were inoculated from each. Definite conclusions cannot be based upon such small numbers.

TABLE XII.

Animal		Dose in mg.	Gain or loss of weight	Killed or died	Duration of life	Post-mortem findings
Buff.	1	50	+109 lb.	K	142 days	Retrogressive tuberculosis.
,,	2	,,	— 18 ,,	D	22 ,,	Broncho-pneumonia.
,,	3	,,	+146 ,,	K	125 ,,	Retrogressive tuberculosis.
,,	4	,,	+ 88 ,,	K	128 ,,	,, ,,
,,	5	,,	— 30 ,,	D	67 ,,	Acute general ,,
,,	6	,,	— 17 ,,	D	101 ,,	,, ,, ,,
,,	7	10	— 4 ,,	K	107 ,,	Retrogressive ,,
,,	8	,,	— 11 ,,	D	39 ,,	Broncho-pneumonia.
,,	9	,,	not weighed	D	3 ,,	Other causes.
,,	10	,,	+ 58 ,,	K	133 ,,	Retrogressive tuberculosis.
,,	11	,,	not recorded.	D	9 ,,	Other causes.
,,	12	Not inoculated. Died from other causes.				
,,	13	,,	,,	,,	,, ,,	
Calf	14	50	+ 64 lb.	K	142 days	Retrogressive tuberculosis.
,,	15	,,	— 8 ,,	K	122 ,,	,, ,,
,,	16	,,	— 23 ,,	D	43 ,,	Acute, general ,,
,,	17	,,	+ 3 ,,	K	122 ,,	Retrogressive ,,
,,	18	,,	— 20 ,,	D	39 ,,	Acute, general ,,
,,	19	,,	— 6 ,,	D	38 ,,	,, ,, ,,
,,	20	10	+ 30 ,,	K	133 ,,	Retrogressive ,,
,,	21	,,	+ 28 ,,	K	128 ,,	,, ,, ,,
,,	22	,,	— 19 ,,	D	37 ,,	Acute, general ,,
,,	23	,,	+ 43 ,,	K	135 ,,	Retrogressive ,,
,,	24	,,	— 7 ,,	D	34 ,,	Acute, general ,,
,,	25	,,	— 12 ,,	D	79 ,,	,, ,, ,,
,,	26	cont.	+ 51 ,,			Control not inoculated.

A comparison of the duration of life in the various guinea-pigs does permit of the inference being drawn that the Ferozepore 118 strain was at least not markedly low in virulence. The facts with regard to the guinea-pigs inoculated with this virus are as follows :—

The guinea-pig inoculated subcutaneously with the original material lived 217 days. This is longer than any other guinea-pig inoculated with original material lived. The duration of life of those inoculated with other strains ranged from 93 to 199 days. On the other hand, the guinea-pig inoculated intraperitoneally died on the 107th day. Only two inoculated intraperitoneally died earlier than this, *viz.*, after 73 and 91 days. Others died as late as the 183rd day. In one instance, specimen 142, the guinea-pig inoculated intraperitoneally, was found to be tuberculous when killed after 271 days. A guinea-pig inoculated with the spleen pulp of this one survived until the 116th day.

Glen Liston's experiments indicated that Indian cattle are less susceptible to infection with the tubercle bacillus than English animals when tested with a virus of European origin.

The experiments here recorded indicate that this is not the sole or possibly even the most important factor in determining the comparatively infrequent occurrence of tuberculosis in Indian cattle. They appear to indicate beyond all possibility of doubt that the strains of tubercle bacilli infecting cattle in India possess a distinctly lower degree of virulence than tubercle bacilli isolated from cattle in Europe.

A point which would appear to support the view that the lower virulence of the organism is the more important factor is that in practically every instance the natural lesions which have come under observation have been restricted to a few glands and have been to a very large extent calcified.

BOVINE LYMPHANGITIS.

BY

A. L. SHEATHER, B.Sc., M.R.C.V.S.,

Director and First Bacteriologist, Imperial Bacteriological Laboratory, Muktesar.

[Received for publication on 6th April, 1920.]

Introductory.

BEFORE describing the experimental work which has been done in connection with the investigation of cases of bovine lymphangitis occurring in the Madras Presidency, it is necessary to consider briefly the accounts which have already been published regarding the occurrence of this disease ; more especially as a disease of this nature has been twice investigated in India during recent years.

The literature on the subject of lymphangitis in cattle appears to be very scanty. Omitting the accounts published during the first half of the nineteenth century, the earliest detailed study of the disease is that by Nocard, who examined specimens from Guadeloupe, and published an article on the subject in the *Annales de l'Institut Pasteur* in 1888.

From this article (*Ann. Inst. Past.*, Vol. 2, 1888, p. 293) it appears that the causal organism of the disease investigated was a streptothrix. This was easily discoverable in pus and in preserved tissues sent to France for examination. It was recovered in culture from original materials and also from animals inoculated experimentally.

The disease is described as starting, as a rule, on the limbs and spreading to the belly. Cording of the lymphatics and enlargement of the glands were noted. This disease was of a very chronic nature.

Nocard found the rabbit resistant to inoculation. In the guinea-pig intraperitoneal or intravenous inoculation caused death in from 9 to 20 days,

and the lesions produced "resembled those of miliary tuberculosis." It is subsequently stated that the thoracic organs were never involved, and that careful examination of the abdominal lesions shewed that they were confined to the peritoneal coverings of the organs, the organs themselves remaining quite healthy.

Subcutaneous inoculation of guinea-pigs lead to the development of an abscess at the seat of inoculation, with involvement of the adjacent glands only, in practically every case.

The organism found could be stained by the Gram-Weigert method but was decolorized by Gram.

The cultural characters were those typical of streptothrix organisms.

The organism retained its virulence for months in culture.

The bacteriological portion of the description of lymphangitis in cattle (Farcin du boeuf) given in Nocard and Lechainche's "Maladies microbiennes des animaux" appears to be based entirely on the account of the disease above referred to.

The clinical description is very largely based upon that published by Maillet in the *Receuil de Mélecine Vé érinaire* in 1837.

The next detailed description of bovine lymphangitis is that given by Vryburg in the *Receuil de Mélecine Vé érinaire*, Vol. 84, 1907, pp. 31, 171, and 241.

The disease described in these articles was observed in Sumatra. It was characterized by the development of abscesses in the skin, connective tissue, lymphatic vessels and glands. The glands usually affected were the prescapular, superficial inguinal, and, more rarely, the popliteal and pubic. There were sometimes lesions in the lungs.

The disease was of a very chronic nature and recovery was the rule. About 10 per cent. of the affected animals died or were killed.

Special note is made of the fact that there was a great tendency to overgrowth of the hoofs owing to the peculiar gait during the later stages of the disease.

As a rule cases were sporadic in herds, but occasionally actual outbreaks were observed. Such outbreaks were spread over long periods, a few animals becoming affected month by month. It would appear from the context that, in many cases at least, draught oxen were the subject of the disease.

Vryburg found that the disease was due to a small bacillus which was present in the pus in pure culture.

He noted particularly that it was difficult to find by means of the microscope, more particularly in old lesions.

The bacillus was easily stained with aniline dyes and sometimes shewed bipolar staining. It was Gram-negative, and somewhat variable in size. It was non-sporulating, a facultative anaerobe, non-gas-forming, and produced indol.

It could be cultivated upon ordinary media, but growth was very slow at first. The addition of serum improved media. No growth was obtained upon potato.

Inoculation of cattle with cultures produced local lesions and abscess-formation in the lymphatic glands. There was no febrile reaction and recovery took place in three months.

Animals which had recovered from natural attacks lasting for periods ranging from 2 months to 3 years failed to become infected when inoculated with cultures.

This agreed with what had been observed under field conditions. A recovered animal seldom became affected a second time.

Pus protected from desiccation retained its virulence for at least a month.

Outside the body the bacillus rapidly lost its virulence and frequent transplantation of subcultures did not prevent this.

Cultures six weeks old seldom produced abscesses when inoculated into cattle, and cultures eight weeks old provoked no reaction at all.

Experiments shewed that subcutaneous inoculation of virulent cultures produced immunity lasting for some months only.

Avirulent cultures produced no immunity.

The blood of infected animals proved sterile in two instances.

A few attempts were made to prepare a protective serum, but the results published are too few to warrant the expression of any opinion as to its value. It appears to be unlikely that a serum would be of any value in so chronic a disease.

Reference is made to the inoculation of a single rabbit only. It is not stated what material was used for the purpose. An abscess developed in a fortnight.

Guinea-pigs inoculated subcutaneously with culture developed abscesses at the seat of inoculation in about three weeks. Intraperitoneal inoculation with culture produced purulent orchitis and death in about two months. The peritoneum near the seat of inoculation shewed small abscesses.

In some cases guinea-pigs inoculated intraperitoneally with culture recovered after suppuration of the testicles had taken place.

Intraperitoneal inoculation of a culture 73 days old produced orchitis and death after two months.

The earlier reference by Vryburg to the rapid loss of virulence of the organism in cultures, must, in view of this statement regarding the infection of a guinea-pig with a culture 73 days old, be held to mean that cultures rapidly lost their virulence for cattle.

The serum of infected animals has very feeble agglutinating powers.

Lymphangitis of cattle in India has been investigated by Raymond and Holmes.

Raymond's investigations began in 1906 and the results were published in 1909 (*Note on Infectious Lymphangitis amongst Draught Bullocks in Calcutta*, Bengal Secretariat Press, Calcutta, 1909).

Holmes commenced his investigations in 1907 and his results were published in the *Journal of Tropical Veterinary Science*, Vol. III, Part 3, 1908, p. 288.

From the details given it would appear that both of these officers were investigating the same outbreak. If that was actually the case, the dissimilarity of the results arrived at is very striking.*

As Holmes' publication antedates that of Raymond, it will be taken first.

Clinically the disease investigated did not differ in any material respect from those described by Nocard and Vryburg.

It was characterized by the appearance of small nodules under the skin of the shoulders, ribs, and flanks, with subsequent involvement of the prescapular and precrural glands. The glands shewed no tendency to burst. There was no rise of temperature until the very last stages. The more severe cases ran a course of about three months, but other cases were far more chronic. In the majority of cases the infection followed a wound of the neck or hump, but in one instance, as in the case of most of the animals referred to by Nocard, the infection appeared to have gained access through a wound in the foot.

The affected glands were found to have thickened fibrous capsules. In some cases the whole of the gland substance had disappeared, the capsule containing thick yellow caseous pus which was smooth to the touch. In other cases the normal tissue remained, but the glands contained a number of small encapsuled abscesses.

* Since this was written I have been able to get into touch with the officer of the Royal Army Veterinary Corps, Lt.-Col. W. B. Edwards, who was in charge of these animals, and have learned from him that Raymond and Holmes did actually investigate the same outbreak independently and in succession. Raymond's investigations were carried out with infected animals handed over to him for the purpose, and Holmes' with materials taken by him in Calcutta and brought to Muktesar for examination.

Where only slight degenerative changes had occurred, the substance w s studded with hard gritty nodules of a yellowish colour resembling the granules of actinomycosis. The pus was "invariably" noticed to be gritty when rubbed between the fingers or slides. (This appears to be in direct contradiction with Holmes' previous statement that the pus was smooth to the touch.—A.L.S.)

In some advanced cases encapsuled abscesses were found in the lungs and bronchial glands.

In every specimen of pus examined Holmes found granules resembling those of actinomycosis.

"Stained with methylene blue, without decolorizing, the masses shewed no structure, except at the periphery, where small individual coccus-like bodies could be distinguished.

By Gram and Gram-Weigert methods the details of the composition of these groups were brought into evidence. They consisted of a dens collection of small cocci, identical in appearance with the spores of actinomy cosis. Closely interwoven with these spores were thin branching filaments.

The filaments were easily decolorized by Gram's method, and were consequently difficult to demonstrate clearly. Unlike the ray fungus there is no concentric (? radial—A.L.S.) arrangement of the filaments, and as a rule no peripheral club formation.

In preparations obtained by scraping the fibrous capsule of the abscesses groups shewing peripheral clubs were found. (It may be here noted that the illustrations do not appear to bear out the descriptions in the text.)

In sections of affected lymphatic glands the same microscopic appearances were seen as in the pus.....Stained by Gram there appeared, scattered throughout the new tissue, the same characteristic dark masses as seen in the pus shewing a dark-stained amorphous centre with cocci and traces of filaments at the periphery.

In many sections masses of filaments alone were found. They were composed of very fine light-stained interlacing threads clearly defined. No cocci or club terminations were present in these latter groups.

This account of the staining reactions and morphology of the organism found by Holmes has been quoted at length because, owing to the somewhat contradictory statements made, it is very difficult to get a clear idea of the appearance of the organism and to make a satisfactory abstract.

Cultures were obtained in about 5 per cent. of tubes inoculated and growth was very slow in the original tubes. Growth took place more rapidly

in subcultures. Passage through rabbits and guinea-pigs caused the organism to grow more rapidly on artificial media.

Aerobic conditions at 37°C. were the most favourable for growth, but growth was obtained between 20° and 37°C., both under aerobic and anaerobic conditions.

On solid media the organism grew with the usual general characters of streptothrix organisms.

In broth a thick greyish white pellicle formed on the surface and subsequently ball-like masses developed at the bottom, the broth remaining clear.

The morphology of the cultivated parasite generally resembled that of the organism as found in the lesions. In old cultures some filaments shewed club-shaped thickenings.

"In other preparations no filaments were present and they consisted of rods and a large ovoid cell taking the stain at the periphery and at either end." This description and the illustration given rather suggest the presence of a large sporulating bacillus as a contamination.

Guinea-pigs inoculated subcutaneously with pus from lesions in cattle developed abscesses at the seat of inoculation only. These shewed no tendency to burst.

Cultures made from such lesions were used for the inoculation of other guinea-pigs and rabbits. The method of inoculation is not mentioned. Death took place in from 2 to 7 days. No mention is made of any lesions being present, but an organism similar to the original culture was isolated from the heart blood.

"Small animals" inoculated intraperitoneally with fresh pus died in from 2 to 6 weeks. In most cases there were no lesions, but from the heart blood of several, pure cultures were obtained.

In two guinea-pigs and three rabbits small abscesses were found scattered over the peritoneum. Cultures from these proved fatal to guinea-pigs and rabbits in a few days and the organism was recovered from the heart blood.

From this it would seem that cultures of the organism produced a septicæmic condition, although it appears to be strange that a pus-producing organism should be capable of infecting guinea-pigs and causing death from septicæmia after a delay of from 2 to 6 weeks without producing any suppurative lesions, as is said to have been the case in guinea-pigs inoculated intraperitoneally with fresh pus.

Two bulls inoculated subcutaneously and two intraperitoneally with pus emulsion failed to react. I have had the opportunity of seeing the charts

of the latter animals, and I find that they were kept under observation for less than a month. This appears to be a very short time in view of the nature of the disease.

One cannot help feeling a little diffidence in accepting Holmes' account of his investigations, as many of the statements made are far from lucid.

The animals which came under Raymond's observation presented the same clinical picture as those seen by Holmes, Vryburg and Nocard.

I quote his desciption.

" All the bullocks which came to me had a wound somewhere on the body. In five instances on the neck, in one on the fetlock, and in three on both neck and fetlock. From these, enlarged lymphatic vessels lead to the prescapular gland, or in the case of the hind leg to the popliteal and precrural glands.

" The affected glands in the older cases when lanced were found generally to contain a large amount of hard cheesy pus encapsuled in hard fibrous tissue. In the neighbourhood of the original wounds, firmly embedded in the skin, some nodules were also observed which contained hard pus. The result of the lancing of the abscesses was a considerable deposit of hard fibrous tissue and the wound took a long time to heal.

" Recurrence was frequent. Glands that were excised for examination were found to consist of a dense fibrous matrix in which purulent foci were distributed. Lameness was present in most of the cases, but general constitutional symptoms, excepting loss of condition, were not always striking, for animals ate their food and performed the usual digestive functions normally.

" The abscesses were in some cases large.

" Two advanced cases which were destroyed at the College (Bengal Veterinary College) were found to have the lungs riddled with encysted abscesses of various sizes up to that of a turkey's egg, the material being nearly always of a hard cheesy sort and the capsule thick."

From such lesions, after a good deal of trouble on account of contaminations, which were largely due to the fact that the work was done in Calcutta during the monsoon, Raymond succeeded in isolating a bacillus which he considered to be the cause of the disease.

Unfortunately he does not give a detailed description of the morphology of the organism, stating only that it was a small bacillus of variable length with rounded ends and that it was not observed to be motile.

With regard to the staining reactions he notes that carbol gentian violet gave the best results and that sometimes a bipolar effect was obtained. It stained with Gram-Weigert but not with Gram.

The organism grew slowly in artificial cultures.

In broth growth was more rapid than on solid media and took a flocculent form which settled to the bottom, the broth remaining clear.

The addition of serum to agar improved it as a culture medium for the bacillus.

On agar very slowly growing greyish white colonies developed.

On gelatin at 20°C. shiny white colonies developed very slowly. The gelatin was not liquefied.

No growth was obtained on potato. Milk was not coagulated. No gas was formed in lactose or glucose broth. Cultures yielded an indol reaction.

The bacillus was strictly aerobic.

Raymond established the bacillus as the cause of the disease by isolating it both direct from natural lesions and after the passage of original pus through guinea-pigs, and reproducing the disease in calves with cultures obtained.

Five strains of the bacillus were isolated in this way.

The guinea-pigs almost invariably shewed suppurative orchitis and abscesses in the omentum and mesenteric glands.

Experimental.

My own investigations have been carried out with materials sent to me by Mr. Ware, Superintendent, Civil Veterinary Department, Madras.

The clinical history of the cases from which the materials were obtained tallies exactly with that given by Raymond.

The first specimens, which were received on November 28th, 1918, were as follows :—

Case I. An abscess removed surgically from the hip of a bull calf aged one year. This had been placed in 10 per cent. formalin immediately after removal.

Two small bottles containing pus which was withdrawn under as complete precautions as are possible in the field from the enlarged precrural gland.

Case II. Pus from an abscess on the shoulder of a six-year-old cow. This animal had had suppuration of one of the precrural glands some time previously. This lesion had been operated upon surgically.

Case I.

Smears of the pus were examined microscopically, but no organisms could be detected.

Tubes of broth and agar were inoculated. Incubation of these for twenty-four hours shewed that a mixture of organisms was present. Further

attempts to isolate the causal organism by direct culture were therefore not made.

Immediately on receipt of the specimen, six guinea-pigs were inoculated subcutaneously (Nos. 3128 to 3133), and six intraperitoneally (Nos. 3134 to 3139). Similarly six rabbits were done subcutaneously and six intraperitoneally (Nos. 1569 to 1574 and 1575 to 1580). At the same time six hill bulls (Nos. 1405, 1406, 1484, 1488, 1509 and 1516) were inoculated subcutaneously on the left side of the neck.

As the results of inoculation of rabbits have less bearing upon the detection of the causal organism than the results obtained with other animals, they will be considered first.

Rabbits inoculated subcutaneously on the inner side of the right thigh with 1 c.c. of emulsion of original pus.

Rabbit 1569. Abscess at the seat of inoculation burst on the 16th day. Second abscess at the seat of inoculation on the 28th day, burst on the 56th day. Killed 34 weeks after inoculation. Perfectly healthy.

Rabbit 1570. Swelling at the seat of inoculation on the 12th day. Disappeared without bursting after 9 weeks. Killed after 34 weeks. Quite healthy.

Rabbit 1571. Abscess at the seat of inoculation on the 15th day. Burst on the 94th day. Killed after 34 weeks. Healthy.

Rabbit 1572. Abscess at the seat of inoculation on the 18th day. Burst on the 39th day. Died on the 64th day. Very poor condition. Muscles on the inner side of the right thigh converted into a thick gelatinous pale yellow pus. Microscopic examination of this revealed an obvious mixture of organisms.

Rabbit 1573. Swelling at the seat of inoculation on the 14th day. Abscess burst on the 133rd day. Second abscess after 5½ months. Opened 14 days later. Killed after 34 weeks. Quite healthy.

Rabbit 1574. Swelling at the seat of inoculation on the 12th day. Abscess burst on the 28th day. Second swelling on the 40th day. Burst on the 62nd day. Killed 27 weeks after inoculation. Quite healthy.

Rabbits inoculated intraperitoneally with **1** *c.c. pus emulsion from Case I.*

Rabbit 1575. Died on the 5th day. Peritonitis. Microscopic examination of the exudate shewed a mixture of organisms.

Rabbit 1576. Killed after 34 weeks. Healthy.

Rabbit 1577. Died of peritonitis on the 16th day. Mixture of organisms present.

Rabbit 1578. Died on the 28th day. Had suffered from diarrhœa. No peritonitis.

Rabbit 1580. Small swelling at the point where the needle had penetrated the abdominal wall which persisted for 18 weeks and then disappeared without bursting. Killed after 27 weeks. Healthy and fat.

From these results it is seen that nothing in the way of a specific reaction could be obtained from the inoculation of rabbits.

Guinea-pigs inoculated subcutaneously with 1 *c.c. of pus emulsion from Case I.*

Guinea-pig 3128. Swelling at the seat of inoculation on the 9th day. Burst on the 16th day. Killed after 33 weeks. Healthy.

Guinea-pig 3129. Swelling at the seat of inoculation on the 13th day. Burst on the 21st day. Killed after 33 weeks. Healthy.

Guinea-pig 3130. Swelling at the seat of inoculation on the 13th day. Killed on the 21st day when swelling was as large as a filbert. At the *post-mortem* this was the only lesion found. The adjacent glands were not involved. Broth and agar cultures made from the pus shewed an obvious mixture of organisms.

Guinea-pig 3131. Swelling at the seat of inoculation on the 4th day. Burst on the 16th day. Killed after 33 weeks. Quite healthy.

Guinea-pig 3132. Swelling at the seat of inoculation on the 4th day. Burst on the 16th day. Killed after 33 weeks. Quite healthy.

Guinea-pig 3133. Swelling at the seat of inoculation on the 4th day. Burst on the 16th day. Killed after 27 weeks. Healthy.

Guinea-pigs inoculated intraperitoneally with 1 *c.c. of pus emulsion from Case I.*

Guinea-pig 3134. Testicles enlarged on the 8th day. Burst and discharged pale yellow pus on the 17th day. Scrotum healed and did not discharge again. Died after 48 days.

Intestines cemented together and to the abdominal wall with a thick pale yellow cheesy material. The parenchyma of the spleen, liver, lungs, and kidneys appeared to be free from lesions of any kind. Testicles entirely converted into a thick pale yellow cheesy pus which was quite smooth when pressed between slides.

No growth was obtained on agar after four days incubation. In broth a small amount of growth was obtained after 4 days. Microscopic

examination of this revealed bacilli which bore some resemblance to the glanders bacillus.

Guinea-pig 3135. Swelling of the testicles on the 8th day. Burst on the 16th day. Scrotum healed. Died after 43 days.

In the left side of the abdomen was an abscess as large as a filbert nut containing cheesy material. The intestines were cemented to this on practically all sides with cheesy exudate.

It was impossible to say where this abscess had originated. A similar abscess appeared to have started in the hepatic glands. The testicles were entirely converted into thick pus. The parenchyma of the liver, lungs, spleen and kidneys was apparently normal. Cultures from the peritoneal exudate proved to be contaminated.

Guinea-pig 3136. Testicles swollen on the 8th day. Burst on the 16th. Died on the 53rd day.

An abscess as large as a golf ball in the left side of the abdomen. Intestines cemented together with cheesy exudate. Testicles entirely converted into pus. Liver, lungs, spleen, and kidney normal.

Guinea-pig 3137. Swelling of the right testicles on the 15th day. Killed on the 20th day.

Suppuration of the tunica vaginalis of the right testicle only. No other lesions of any kind. Cultures in broth obtained.

Microscopic examination revealed a bacillus resembling that of glanders in a state of purity.

It may here be noted that a subculture was obtained from one of the original broth tubes, which were inoculated on December 18th, 1918, after they had been kept at room temperature for a year, on December 31st, 1919. This strain is still under cultivation.

Guinea-pig 3138. Swelling of the testicles on the 11th day. Killed on the 18th day.

Suppuration of the tunica vaginalis in both halves of the scrotum, and five millet seed abscesses in the omentum.

Pure cultures obtained in broth.

Guinea-pig 3139. Swelling of the testicles on the 14th day. Burst on the 32nd day. Killed on the 42nd day.

Both testicles converted into thick pale yellow pus. On the left side of the abdomen all the intestines were cemented together with thick yellow exudate, and scattered over the surface of the visceral peritoneum in other parts were discrete collections of pus varying in size from a pea to a small nut. These appeared to have been formed under the peritoneal membrane, which

acted as a capsule. The gastric glands were about the size of lentils and caseous. Cultures proved to be contaminated.

Guinea-pigs inoculated intraperitoneally with 1 *c.c. of second culture from Guinea-pig* 3137.

This culture was made from a primary broth tube after the latter had remained at room temperature for just over a year.

Guinea-pig 3. Swelling of the right testicle on the 33rd day after inoculation. Scrotum burst and discharged on the 39th day. Killed on the 41st day.

The right testicle was found to be adherent to the base of the scrotum, but there was no visible pus. Otherwise quite healthy.

Guinea-pig 4. This animal's temperature fell from the date of inoculation and it died without shewing any lesions on the 11th day.

Bulls inoculated subcutaneously on the left side of the neck with 5 *c.c. of pus emulsion from Case I.*

Hill bull 1405. Three-and-a-half years old. A fortnight after inoculation a swelling developed at the seat of inoculation, and a week later the prescapular gland on the same side shewed slight enlargement. The local lesion was opened four weeks after inoculation and from the pus a pure growth of a bacillus somewhat resembling the glanders bacillus was obtained.

The gland steadily decreased in size until it was normal two months after inoculation.

The bull was subsequently kept under observation for seven-and-a-half months and failed to shew any further evidence of infection.

For several months during 1919 there was a shortage of bulls for the production of rinderpest virus, and this animal was therefore utilized for the purpose. A typical rinderpest reaction was obtained, and at the *post-mortem* examination only the lesions of rinderpest were found.

Four-and-a-half weeks after the original inoculation a little pus obtained from the local lesion was rubbed into a small sore on the quarter. No infection resulted.

Hill bull 1406. Three years old. The history of this animal is identical with that of No. 1405 with the exception that it was kept under observation for a period of three months after all trace of swelling of the prescapular gland had disappeared. It was then used as a rinderpest virus-producer. At the *post-mortem* only lesions of rinderpest were found.

Hill bull 1484. The clinical history of this animal is the same as that of 1406, but when it was killed a little encapsuled collection of pus about half an inch in diameter was found in contact with the capsule of the prescapular gland on its inner surface. The gland substance itself shewed no suppuration. Cultures in broth shewed a bacillus exactly like that isolated from the other experimental animals.

Hill bull 1488. A very small abscess developed at the seat of inoculation which was opened after one month. No cultures were made. There was slight enlargement of the prescapular gland which had disappeared five weeks after inoculation. From this time onwards the animal was kept under observation for ten-and-a-half months without shewing any evidence of infection. It was then used for rinderpest virus and, when killed, shewed the lesions of rinderpest only.

Hill bull 1502. In this animal a small swelling developed at the seat ot inoculation and its contents were evacuated five weeks after inoculation. Cultures were not made. The prescapular gland was just appreciably enlarged from the third to the fourth week after inoculation. After the abscess had been evacuated the animal was kept under observation for eight-and-a-half months when it was used for rinderpest virus. When it was killed only the lesions of rinderpest were found.

Hill bull 1516. Abscess-formation at the seat of inoculation developed more promptly in this animal as the local lesion burst spontaneously after a fortnight. There was a transitory slight enlargement of the prescapular gland from the third to the fourth week. Eight-and-a-half months after when all swelling of the gland had disappeared the animal was used as a rinderpest virus-producer. Only lesions of rinderpest were found at the *post-mortem*.

Case II.

Pus from an abscess on the shoulder of a six-year old cow. This animal had had suppuration of one of the precrural glands for some time previously. This lesion had been operated upon surgically. Microscopic examination of the specimen of pus shewed that it contained a variety of types of organisms in small numbers. In view of this fact it was decided that experimental inoculation from it would only entail, in all probability, the useless sacrifice of a considerable number of small animals.

The inoculation of broth and agar tubes proved immediately that the pus was heavily contaminated.

No further experiments were carried out with this specimen.

It is unfortunate that when the investigation had reached this point circumstances combined to put an end to the work for the time being. The monsoon had failed and grass was difficult to obtain, influenza and relapsing fever broke out and caused the coolies and cattle-men to run away in such numbers that for some weeks it was scarcely possible to feed the animals required for serum-making. Added to this there was an immensely increased demand for anti-rinderpest serum. The routine and administration work entailed in overcoming these difficulties precluded any possibility of any research work being done, more especially as there was simultaneously a shortage of officers.

Some valuable information for future guidance was, however, obtained from the somewhat scanty work done. This may be summarized as follows :—

The outstanding feature is the production of suppurative orchitis in the guinea-pigs inoculated into the peritoneum. This is of value in confirming Raymond's and Vryburg's results.

It is a somewhat striking fact that a certain proportion of guinea-pigs so inoculated recover completely except for the damage done to the testicles. The bacillus which is apparently the cause of the disease was isolated from lesions produced in guinea-pigs and bulls inoculated with original pus. This bacillus was capable of producing orchitis in guinea-pigs by intraperitoneal inoculation. A culture of this bacillus retained its vitality, and at least a proportion of its virulence for the guinea-pig, when subcultured after remaining for a year at room temperature. A few points were noted in a general way when time was available for laboratory work. It was not found possible to stain the bacillus isolated by any of the complex methods. It was negative to Gram, Gram-Weigert and Claudius, but it could be well stained with methylene blue, carbol fuchsin, and carbol gentian violet. One per cent. acetic acid could be used for decolorizing stained debris of culture medium in preparations without materially decolorizing the bacilli.

The bacillus was very difficult to detect in smears from the original pus, in sections from the original abscess, and in smears from some of the experimentally inoculated animals. In some cases the bacilli could be found with comparative ease in films of pus from cases of orchitis in the guinea-pig. The difficulty of detecting the bacilli was partly due to the fact that the organisms were present in very small numbers, and partly to the fact that differential methods of staining could not be applied. Microscopic examination of the pus would therefore play a negligible part in diagnosis.

The bacillus was found to grow very slowly in primary cultures. Broth appeared to be the most favourable medium for the purpose. Subcultures

could be obtained on serum agar and serum glycerine agar, and from these on to ordinary agar.

In broth the growth took a flocculent form which settled to the bottom, the broth remaining clear. A good deal of shaking was required to disturb the growth, and the flocculi could not be broken up without considerable force being used. In subcultures on agar and serum agar the growth took the form of discrete colonies which were translucent and of a slightly yellowish tint by transmitted light, and greyish white and glistening by reflected light.

No growth was obtained on potato.

With successive subcultures the period of incubation required to produce a visible growth rapidly became shorter, and by the fourth generation colonies were visible in 18 hours. In subcultures which had been sown freely a continuous layer of growth was obtained during the second 24-hour incubation. Opportunity did not offer for a detailed examination of the morphology of the bacillus, but it would appear from such examination as could be made that it is somewhat pleomorphic, its morphology varying with age and the nature of the culture medium used.

These findings agree very closely with those of Raymond and Vryburg and are absolutely at variance with those of Holmes and Nocard.

In no single instance was any evidence of a streptothrix organism discovered in any of the materials examined.

The animal inoculation and artificial culture results are also entirely different from those obtained by the latter authors.

This would be susceptible of the simple explanation that the two groups of investigators had under examination different diseases, but difficulty arises in view of the fact that in the published records there is every indication that Raymond and Holmes actually investigated the same outbreak in succession.

Cases III and IV.

In July 1919 a fresh supply of specimens from cases of bovine lymphangitis was received from Madras.

These comprised: Case III (*a*) pus collected aseptically from the prescapular gland of a bull three years old. Both prescapular glands and both precrural glands had been opened previously, but pus was again forming in these situations as well as "in other parts of the body." (*b*) Left prescapular gland of the same animal preserved in formalin.

Case IV. Pus from an abscess in the left popliteal gland of a cow five years old. The pharyngeal and precrural glands of this animal were also enlarged and indurated.

The specimens were received on July 24th, 1919.

In the light of the experience gained from the previous experiments it was decided that no attempt should be made to obtain primary cultures from the original pus.

Eight guinea-pigs and eight rabbits were inoculated from each of the specimens III and IV, four subcutaneously and four intraperitoneally.

In view of the somewhat unsatisfactory nature of the results obtained by the inoculation of hill bulls with the previous specimen plains calves were used in this instance, and six, Nos. 14 to 19, were inoculated subcutaneously on the left side of the neck with an emulsion of pus on August 13th. The delay in inoculating these calves was due to the necessity of getting them up from the plains, as none were available in Muktesar. Nos. 14 and 15 were done from Case III and the remainder from Case IV.

As in the previous case the rabbits will be dealt with first. Rabbits 41 to 48 were inoculated with 1 c.c. of an emulsion of pus from Case III on July 28th, 1919. Of these, 41 to 44 were done intraperitoneally and 45 to 48 subcutaneously on the inner side of the right thigh. The whole of these were under observation for a period of six months without shewing the slightest disturbance of health.

Rabbits inoculated with pus from Case IV.

Rabbits 49 to 56 were inoculated with 1 c.c. of pus emulsion from Case IV, 49 to 52 subcutaneously and 53 to 56 intraperitoneally.

Rabbit 49. Shewed a swelling at the seat of inoculation at the end of a week. This increased in size very slowly and burst six weeks later. The animal was kept under observation for four-and-a-half months without shewing any further evidence of infection.

Rabbit 50. Swelling at the seat of inoculation on the 4th day. Abscess burst on the 14th day and the animal completely recovered. It was under observation for six months.

Rabbit 51. Exactly as No. 50.

Rabbit 52. The same as No. 50.

Rabbit 53. Shewed no disturbance of any kind. Under observation for six months.

Rabbit 54. Shewed no abnormalities for three months after inoculation. The temperature then fell rather suddenly from the average of 37·5° to 35°or less, and it oscillated between this and 37° for five weeks. The animal then died. No lesions were discoverable at the *post-mortem*.

Rabbit 55. Shewed no disturbance of any kind. Under observation for six months.

Rabbit 56. Under observation for six months. No disturbance of health.

Guinea-pigs **147—150** *inoculated subcutaneously with* 1 *c.c. of pus emulsion from Case III.*

Nos. 147 to 150 were inoculated subcutaneously on the inner side of the right thigh with 1 c.c. of pus emulsion.

Guinea-pig 147. Kept under observation for seven months. No disturbance of health. In *post-mortem* no lesions of any kind were found.

Guinea-pig 148. Swelling at the seat of inoculation after 14 days. Burst on the 20th day. Animal kept under observation for seven months. Healthy.

Guinea-pig 149. No local lesion of any kind, but died on the 41st day. Pneumonia.

Guinea-pig 150. Developed a small hard swelling at the seat of inoculation on the 17th day. This was reabsorbed without bursting. Killed after seven months. Healthy.

Guinea-pigs 151—154 *inoculated intraperitoneally with* 1 *c.c. of pus emulsion from Case III.*

Guinea-pig 151. Enlargement of the testicles on the 16th day. The swelling burst on the 25th day. Wound healed. Kept under observation for seven months. No lesions except testicles rather reduced in size and adherent to the base of the scrotum.

Guinea-pig 152. Enlargement of both testicles on the 17th day. Found dead on the 29th day. Both testicles entirely converted into pus and a single abscess as large as a pea in the omentum.

Guinea-pig 153. Both testicles enlarged on the 17th day. Burst on the 39th day. Killed four months after inoculation. Slight adhesion of both testicles to the base of the scrotum only.

Guinea-pig 154. Enlargement of the testicles on the 10th day. Killed on the 26th day. Skin over the left half of the scrotum burst while being cleansed for *post-mortem*. At the point of penetration of the needle an abscess as large as a pea; an abscess as large as a horse bean in both mesentery and omentum. Spleen mottled with pale yellow irregular patches. Kidneys, liver, lungs and peripheral glands normal. Cultures in broth and serum agar from testicular pus contaminated.

Guinea-pigs 155—158 *inoculated subcutaneously with* 1 *c.c. of pus emulsion from Case IV.*

Guinea-pig 155. Swelling of both testicles on the 16th day. Scrotum burst on the 25th day. Wound healed. Animal died on the 85th day. Slight adhesion of both testicles to scrotum only.

Guinea-pig 156. No disturbance of health. Under observation for seven months.

Guinea-pig 157. Abscess at the seat of inoculation on the 16th day. Burst on the 25th day. Complete recovery. Killed after seven months. Healthy.

Guinea-pig 158. Under observation for seven months. Perfectly healthy.

Guinea-pigs 159—162 *inoculated intraperitoneally with* 1 *c.c. of pus emulsion from Case IV.*

Guinea-pig 159. Enlargement of left testicle on the 22nd day. Burst on the 30th day. Killed four months after inoculation. Quite healthy except for adhesion of the left testicle to the base of the scrotum.

Guinea-pig 160. Swelling of both testicles on the 22nd day. Left side of scrotum burst on the 30th day and the right side on the 38th day. Killed seven months after inoculation. Slight adhesion of testicles to the base of scrotum only.

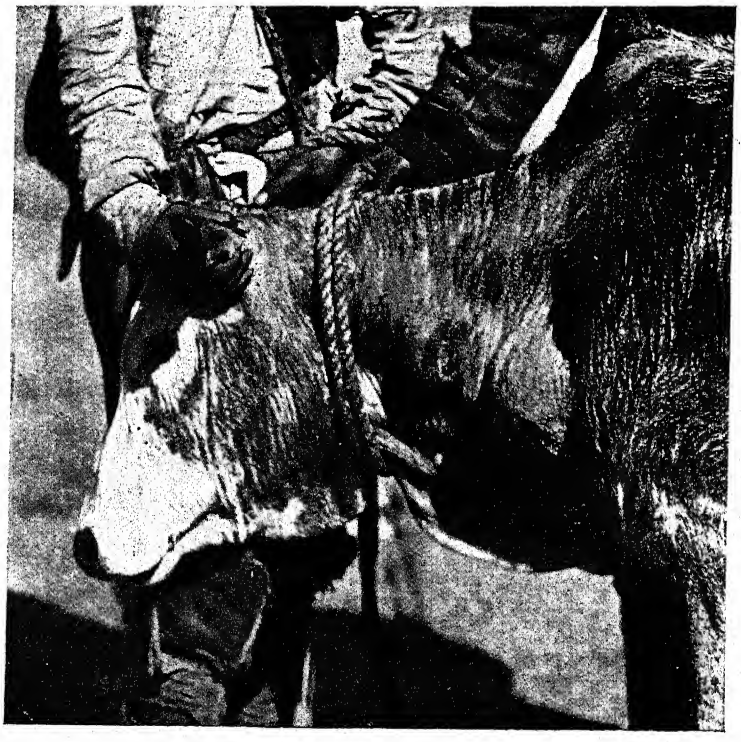
Guinea-pig 161. Swelling of testicles on the 15th day. Burst on the 28th day. Died seven-and-a-half weeks after inoculation. No lesions of any kind.

Guinea-pig 162. Swelling of testicles. Burst on the 34th day. Recovered.

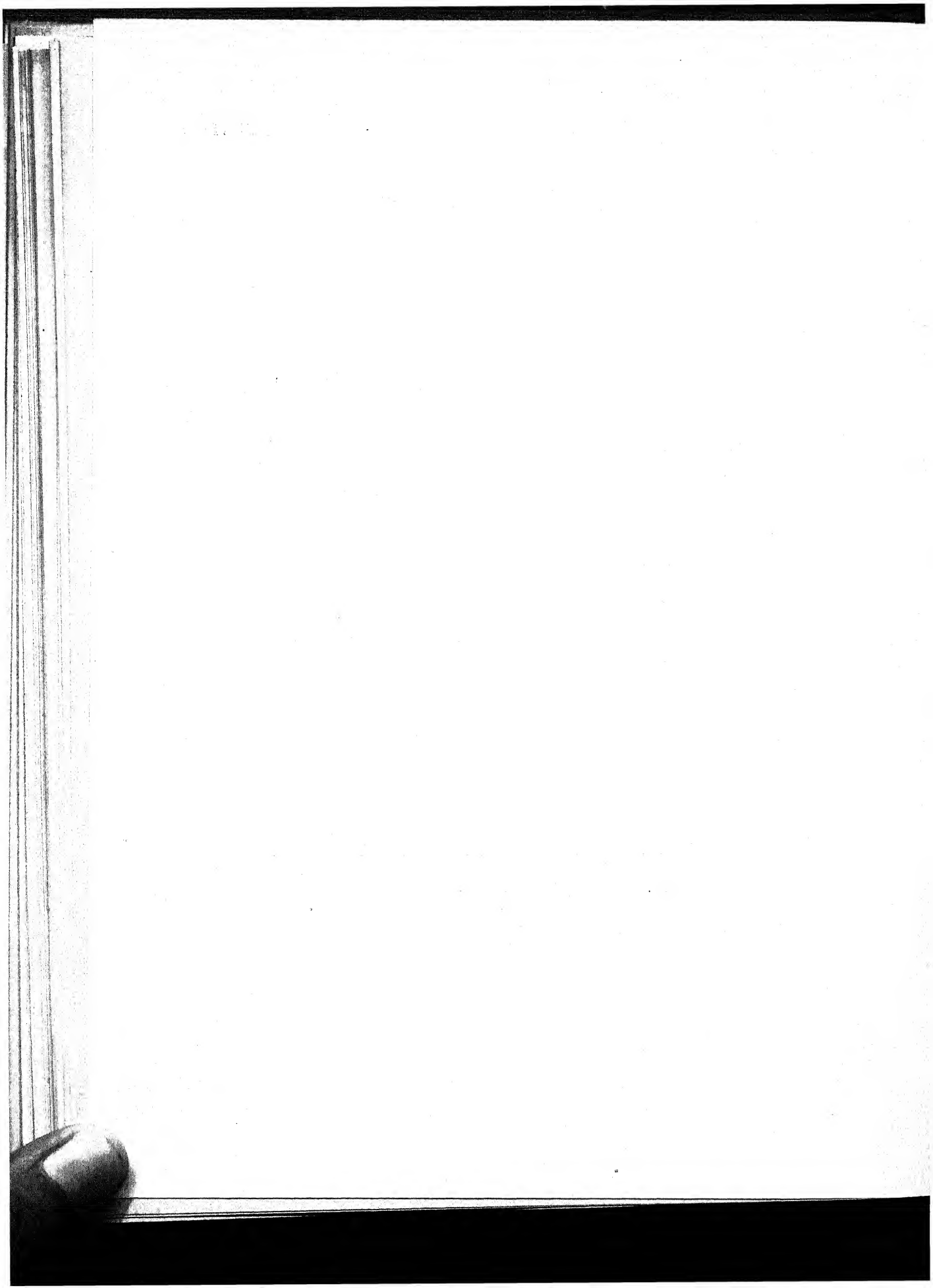
Calves inoculated subcutaneously on the left side of the neck with 3 *c.c. of pus emulsion from Case III.*

Plains calf 14. The day following inoculation there was a small swelling at the seat of inoculation which steadily increased in size up to that of an orange. Six weeks after inoculation this shewed signs of pointing and was opened.

The prescapular gland on the same side shewed slight enlargement five days after inoculation; this increased until it attained the size of an orange. Thirteen weeks after inoculation the gland was tapped and a little pus obtained for further experiments. (Plate I.)



Calf 14. 22nd September, 1919.
Shewing local lesion and enlarged prescapular gland.
Original pus inoculation. (Case III.)



Plains calf 15. The day after inoculation there was a small swelling at the seat of inoculation. A few days later the prescapular gland shewed a slight degree of enlargement. Both swellings disappeared in the course of the next five weeks. Six weeks after the original inoculation the calf was reinoculated on the right side of the neck with an emulsion of pus obtained from the local lesion of Calf 14. Swelling at the seat of inoculation and of the right prescapular gland followed. The abscess at the seat of inoculation burst six weeks later. The orifice closed but suppuration occurred again after a further interval of twelve days.

On December 13th, seventeen-and-a-half weeks after inoculation, the right prescapular gland was tapped and pus obtained for further examination.

Plains calves inoculated on the left side of the neck with emulsion of pus from Case IV.

Plains calf 16. A slight transient swelling at the seat of inoculation only.

On September 22nd the animal was reinoculated on the right side of the neck with an emulsion of the pus obtained from the local lesion of Calf 14 on the same date.

Eight weeks after this inoculation a local lesion as large as a duck's egg burst. The right prescapular gland had by this time attained the size of an orange. Pus was obtained by tapping the gland on December 18th, about three months after inoculation.

Plains calf 17. A transient local swelling and enlargement of the prescapular gland followed the inoculation of original pus. Six weeks after the first inoculation the animal was again inoculated on the opposite side of the neck with an emulsion of pus obtained from the local lesion of Calf 14.

A small abscess slowly matured at the seat of inoculation and burst after eight weeks, when it was the size of a hen's egg. The prescapular gland attained the size of an orange, but up to the time of writing had shewn no tendency to point.

Plains calf 18. The original inoculation produced only a transient swelling locally.

Plains calf 19. Like Calf 18.

Guinea-pigs inoculated with pus obtained from the local lesion of Calf 14.

Guinea-pig 210. Inoculated intraperitoneally. Died on the 13th day. The animal's temperature fell more or less regularly from the date of inoculation. No lesions.

Guinea-pig 211. Intraperitoneal. Enlargement of the testicles during the third week. Killed on the 23rd day. Suppuration of the tunica vaginalis in both halves of the scrotum. Five abscesses as large as lentils in the omentum.

Guinea-pig 212. Like No. 211, but suppuration of the tunica vaginalis only.

Guinea-pig 213. Failed to shew any evidence of infection. Kept under observation for five months.

Guinea-pigs inoculated intraperitoneally with pus obtained by tapping the prescapular gland of Calf 14.

Guinea-pig 243. Left testicle swollen on the 16th day. Killed on the 25th day. Left testicle entirely converted into pus. One abscess as large as a pea in the omentum.

Guinea-pig 244. Shewed no evidence of infection. Killed three months after inoculation. Healthy.

Guinea-pig 245. Left testicle swollen on the 19th day. Burst on the 26th day.

Eleven weeks after the original inoculation an abscess developed on the abdominal wall. This attained the size of a marble in the course of a fortnight. Killed thirteen weeks and three days after inoculation. The left testicle was somewhat reduced in size, but there was no visible pus in the scrotum. Except for the abscess on the abdominal wall the animal was healthy.

Guinea-pig 246. Swelling of the testicles on the 16th day. Both sides of the scrotum burst and suppurated on the 32nd day. The animal died eight-and-a-half weeks after inoculation. The scrotum had healed.

Both testicles entirely converted into thick pale yellow pus. The lymphatics of the spermatic cords contained pus for a distance of about an inch. The mesentery contained three thinly encapsuled abscesses as large as marbles. From the position of these it appeared that they might have originated in the mesenteric glands.

Guinea-pig 247. Right testicle enlarged on the 29th day. Killed on the 38th day. Right testicle converted into pus. No abdominal lesions.

Guinea-pig 248. Died without shewing lesions on the 13th day.

Guinea-pigs inoculated with pure broth culture obtained from the pus aspirated from the prescapular gland of Calf 14.

Guinea-pig 249. Right testicle enlarged on the 6th day. Killed on the 14th day.

Right half of the scrotum contained pus, but the testicle itself was not involved. In the left side of the abdomen the intestines were cemented together and to the abdominal wall with yellow exudate.

Guinea-pig 250. Died on the 6th day. Omentum contained about a score of abscesses varying in size up to that of a lentil. In each half of the scrotum there were two collections of pus about the size of oat grains.

Guinea-pig 251. Enlargement of the testicles on the 10th day. Killed on the 19th day. Both testicles entirely converted into pus.

Intestines cemented together with thick yellow exudate.

Guinea-pig 252. Testicles swollen on the 9th day. Killed on the 17th day. Both testicles entirely converted into pus. Intestines cemented together with yellow exudate.

Guinea-pigs inoculated intraperitoneally with pus from the local lesion of Calf 15.

Guinea-pig 273. Shewed no evidence of infection during life. Died five weeks after inoculation. Enteritis.

Guinea-pig 274. Swelling of the right testicle on the 18th day. Scrotum suppurated on the 27th day. Killed on the 56th day. Right testicle about half normal size, left normal. Omentum contained a thin-walled abscess about as large as a walnut. No other lesions.

Guinea-pig 275. Swelling of the testicles on the 14th day. Opened scrotum under strictest precautions on the 17th day and obtained pus for cultures, etc. Died on the 29th day. Scrotal wound healed, no pus in scrotum. Two small encapsuled abscesses in omentum, purulent pericarditis and congestion of the lungs.

Guinea-pig 276. Died without having shewn any evidence of infection on the 7th day. No lesions.

Guinea-pigs inoculated intraperitoneally with pus obtained by puncture of the prescapular gland of Calf 16.

Guinea-pig 277. Swelling of the testicles on the 15th day. Opened under strictest precautions on the 24th day. Killed eight weeks after inoculation. Left testicle entirely converted into pus. Right testicle entirely gone. (This half of the scrotum had been opened.) Left inguinal gland as large as a horse bean and suppurating. Left precrural gland slightly enlarged and containing pus. Omentum contained three encapsuled abscesses as large as marbles.

Guinea-pig 278. Swelling of the testicles on the 12th day. Material for culture obtained on the 20th day. Died on the 33rd day.

Suppuration had occurred in both sides of the scrotum, and the testicles, which were reduced in size, were adherent to its base. Omentum contained three abscesses as large as marbles.

Guinea-pig 279. Failed to shew any evidence of infection and was perfectly healthy when killed two months after inoculation.

Guinea-pig 280. Enlargement of testicles on the 14th day. Scrotum burst on the 19th day. Killed two months after inoculation.

A thin layer of necrotic tissue about half an inch wide running along the linea alba, surrounded by a small amount of gelatinous exudate. Mesentery contained an abscess as large as a filbert nut.

Guinea-pigs and calves inoculated with cultures obtained from pus from the prescapular gland of Calf 16.

The cultures used were broth cultures incubated 90 hours.

Guinea-pigs received 0·5 c.c. intraperitoneally and the calves 3 c.c. subcutaneously on the left side of the neck.

Guinea-pig 281. Swelling of the testicles on the 12th day. Scrotum opened under strict precautions on the 31st day. Killed nine weeks after inoculation. Both testicles about one-quarter the normal size. Extensive adhesion between the omentum, liver, and abdominal wall, but no evidence of exudate.

Guinea-pig 282. Right testicle swollen on the 28th day. Burst on the 33rd day. Killed nine weeks after inoculation. Right testicle about one-third normal size and adherent to the scrotum. No pus.

Guinea-pig 283. Swelling of the testicles on the 16th day. Burst on the 26th day. Killed after nine weeks. Both testicles reduced in size and adherent to the base of the scrotum.

Guinea-pig 284. Swelling of the left testicle on the 13th day. Burst on the 27th day. Killed after nine weeks. Left testicle much reduced in size and adherent to the base of the scrotum. No other lesion.

Guinea-pig 285. Swelling of testicles on the 9th day. Scrotum opened under strict precautions on the 24th day. Killed after nine weeks.

Both testicles about one-quarter the normal size. No pus.

Guinea-pig 286. Swelling of the testicles on the 9th day. Opened on the 24th day. Killed after nine weeks. Omentum contained four encapsuled abscesses varying in size from a bean to a walnut. In the left half of the scrotum there was a collection of pus as large as a pea. Both testicles were about one-quarter the normal size and adherent to the base of the scrotum.

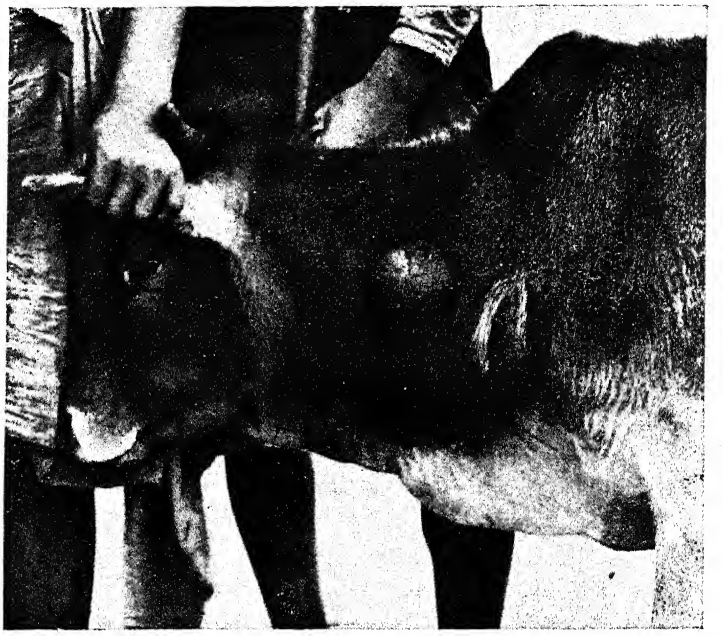
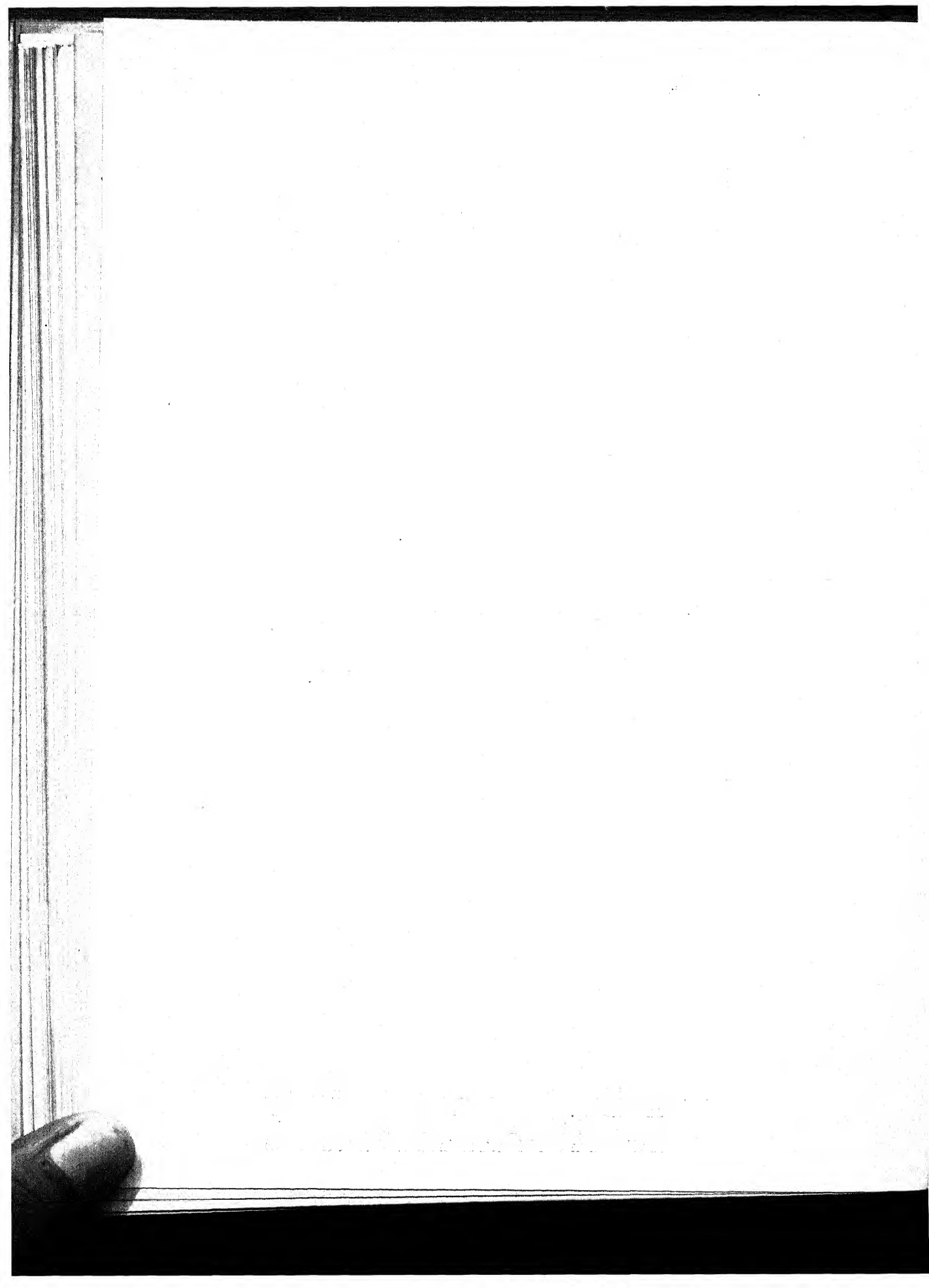


Fig. 1. Calf 23. 25th February, 1920.
Shewing local lesion and enlarged prescapular gland. Inoculated with culture derived from the prescapular gland of Calf 16. The skin round the gland has been wetted to render the enlargement more visible.

Fig. 2. Bacilli in film of pus from prescapular gland of Calf 21 (×2000). (This and all the other preparations of the bacilli shewn were stained with the special modification of Neisser's stain referred to in the text.)



Plains calf 20. A small swelling at the seat of inoculation which shewed evidence of pointing during the 4th week. Pus was obtained from this under strict precautions on the 26th day. The prescapular gland shewed some enlargement on the 19th day. This has persisted with little or no alteration up to the time of writing, ten weeks after inoculation.

Plains calf 21. History as No. 20. A little pus was obtained from the local lesion on the 18th day.

Plains calf 22. The inoculation was followed by the development of an abscess, which was tapped on the 21st day, and by enlargement of the prescapular gland from the 18th day. The gland was tapped six weeks after inoculation and pus obtained for further experiment.

Plains calf 23. Pus was obtained from the local lesion 24 days after inoculation. The prescapular gland shewed very slowly progressive swelling from the 18th day. (Plate II, fig. 1.)

Plains calf 24. Pus was obtained from the local lesion 35 days after inoculation. The prescapular gland shewed enlargement from the 19th day onwards.

Plains calf 25. The prescapular gland shewed enlargement from the 19th day. The swelling at the seat of inoculation attained the size of a turkey's egg, but shewed no signs of bursting.

Guinea-pigs inoculated intraperitoneally with 48-hour broth culture from the testicular lesion of Guinea-pig 275 *which had been inoculated intraperitoneally with pus from Calf* 15.

Guinea-pig 1. Both testicles swollen on the 4th day. Swelling opened on the 16th day. Killed seven weeks after inoculation.

One encapsuled abscess as large as a marble in the omentum and another about half as large again on the course of the lymphatics leading from the right testicle. Both testicles very small and adherent to the base of the scrotum.

Guinea-pig 2. Was dull on the 4th day. On the 5th day the testicles were swollen. The animal died the following night.

A trace of pus was found in both halves of the scrotum.

Before considering in detail the organism isolated from the various lesions referred to the results of the inoculations may be correlated and criticized.

Results obtained with rabbits.

The results obtained in rabbits will be dealt with first.

With the exception of one, No. 1572, all the rabbits inoculated subcutaneously with the original pus of Case I developed an abscess at the seat of

inoculation which burst in most cases. Complete recovery followed. No. 1572 shewed deep suppuration.

Of the six rabbits inoculated intraperitoneally with the same material, three died of peritonitis, one of enteritis, and the remaining two failed to become infected, save that one shewed a small local infection which did not mature.

All the eight rabbits inoculated from Case III remained healthy and were under observation for six months.

Four rabbits inoculated intraperitoneally from Case IV remained perfectly healthy. The four done subcutaneously developed local lesions which burst and healed.

In view of the knowledge that some at least of the materials used contained impurities, in the absence of any knowledge as to the possible pathogenic properties of such impurities, and in view of the positive results obtained in guinea-pigs with that particular specimen, the results obtained from the inoculation of rabbits with material from Case III must be considered the most important.

The conclusion to be drawn is that the rabbit is not susceptible to infection with the organism responsible for the original disease.

The results obtained with guinea-pigs.

The six guinea-pigs inoculated subcutaneously with pus from Case I developed local lesions only, and all recovered.

All the guinea-pigs, six in number, inoculated intraperitoneally with pus from Case I developed a suppurative orchitis, or, more correctly speaking, a suppurative lesion of the tunica vaginalis of the scrotum with or without subsequent involvement of the testicles, and abscesses formed in the peritoneal cavity.

The development of orchitis, using the term in its wider sense, in two guinea-pigs (Nos. 3 and 4) which were inoculated intraperitoneally with culture derived from the testicular lesion of a guinea-pig itself inoculated with original pus, is important.

Of the four guinea-pigs inoculated subcutaneously with pus from Case III, two failed to become infected, one developed a local lesion which was reabsorbed without bursting, and one developed a local lesion which burst, the animal making a complete recovery.

All the guinea-pigs inoculated intraperitoneally developed suppurative orchitis with or without lesions in the abdomen.

Four guinea-pigs were inoculated subcutaneously with pus from Case IV. Two of these remained healthy, one developed a local lesion and recovered, and the remaining one developed orchitis.

All the four which were inoculated intraperitoneally developed orchitis.

RESULTS OBTAINED IN CALVES.

Calves inoculated from Case I. All the animals developed local lesions associated with transient enlargement of the prescapular gland. In only one instance, No. 1484, was there any evidence of persistent infection when the animal was destroyed ; this, however, was very slight.

Broadly speaking, these results from the point of view of lending any assistance of value for the detection of the causal organism must be considered negative.

It is true that the local lesions may have been due to the organism responsible for the original disease, the smallness of the reaction being due to natural resistance possessed by hill (Kumaon and Garhwal) bulls.

This possibility was taken into account when the second batch of specimens (Cases III and IV) arrived. For the experiments with these, plains bulls were obtained specially from the plains (Bareilly District).

Calves inoculated from Cases III and IV. Of the two calves (Nos. 14 and 15) inoculated from Case III, one (No. 14) developed an abscess at the seat of inoculation and pus was obtained from the prescapular gland. The other shewed only transient swellings. When this calf was reinoculated with pus from the local lesion of Calf 14 a local lesion was produced, and pus was obtained from the prescapular gland. None of the four calves inoculated from Case IV developed any lesions.

Two of these (Nos. 16 and 17) when reinoculated with pus from the local lesion of Calf 14 developed lesions at the seat of inoculation, and pus was obtained from the prescapular gland of No. 16.

Nos. 18 and 19 were not inoculated a second time in order to see whether they would subsequently develop lesions although the inoculations had apparently caused only transient effects.

In view of the fact that only Calf 14 out of the six used reacted to inoculation with original material from Cases III and IV, it might be held that the results obtained with plains calves were no more satisfactory than those obtained with hill calves. But when the results of the second inoculations are considered one is justified in concluding that the escape of Calves 15 to 19 was in the first instance more or less in the nature of an accident.

If the accidental nature of this escape is allowed then the second inoculation with pus from Calf 14 was almost tantamount to a reinoculation with

original pus, the only modifying factor being the single passage through a plains calf.

It must be concluded, tentatively at least, that hill animals do possess a higher degree of resistance than plains animals.

Summary of Results.

Summarizing the results of inoculation with original pus the following are the chief points which emerge:—

1. Rabbits are not susceptible to infection.
2. Guinea-pigs inoculated intraperitoneally develop purulent orchitis in a very large percentage of cases.
3. Susceptible cattle inoculated subcutaneously develop a local lesion with involvement of the nearest gland.

It may be here noted that methods other than subcutaneous inoculation were not used for the infection of cattle because that would appear to approach more closely to the natural method of infection.

There now come for consideration the animal experiments which were carried out with materials other than original specimens of pus.

As already mentioned, pressure of other duties put a stop to the investigations which were started in 1918, and of the materials used there remained, when Cases III and IV came for investigation, only one original culture. This was a broth culture from the testicular lesion of Guinea-pig 3137 which had been inoculated intraperitoneally with the original pus of Case I. A subculture of this broth culture was found to be virulent for the guinea-pig with the production of the same lesions as those caused by inoculation with original and subsequent specimens of pus, and cultures ultimately obtained from these.

This culture has a certain value, as it furnished indications as to the length of time that a particular bacillus will retain its vitality and at least a certain degree of its virulence for the guinea-pig. The constancy of the lesions produced indicated that the bacillus is causally connected with the disease under investigation.

The lesions developed by Calves 14, 15, and 16 formed the starting point of the second group of animal experiments.

Of the four guinea-pigs inoculated intraperitoneally with pus from the local lesion of Calf 14, two developed purulent orchitis, one died rather soon after inoculation without developing lesions, and the remaining one remained under observation for five months without shewing any evidence of infection.

Of the six guinea-pigs inoculated intraperitoneally with pus withdrawn from the gland of Calf 14, four developed purulent orchitis, one was under observation for three months without shewing evidence of infection, and one died 13 days after inoculation.

All the guinea-pigs inoculated intraperitoneally with culture derived from the pus from the gland of Calf 14 developed purulent orchitis, with abdominal lesions in addition.

Of the four guinea-pigs inoculated intraperitoneally with pus from the prescapular gland of Calf 15, one died without developing lesions, one was kept under observation for five weeks without shewing evidence of infection, and then died, apparently from enteritis, and the remaining two developed purulent orchitis, with abdominal lesions.

Three of the guinea-pigs inoculated with pus from the prescapular gland of Calf 16 shewed orchitis and abdominal lesions. One shewed no evidence of infection.

All the six guinea-pigs inoculated with culture from this pus developed purulent orchitis with or without other lesions.

Six calves inoculated with the same culture all developed more or less pronounced local lesions with involvement of the gland.

There remain two guinea-pigs inoculated with culture derived from the testicular lesion of a guinea-pig which had been inoculated with pus from the local lesion of Calf 15. Both of these developed purulent orchitis.

From the constancy of the lesions produced with materials from both batches of specimens it would appear that the organism which has been isolated from both is actually the cause of the original disease.

The following strains were isolated and proved constant in their characters :—

1. The sole remaining culture from the first batch of specimens which was isolated from the testicular lesion of Guinea-pig 3137.
2. Strains from the testicles and omentum of Guinea-pigs 243, 246, and 247, which had been inoculated from the prescapular gland of Calf 14.
3. Strains from the testicles of Guinea-pigs 249, 251 and 252 which had been inoculated with culture derived from the prescapular gland of Calf 14.
4. Testicles of Guinea-pig 275 inoculated from the local lesion of Calf 15.
5. Testicles of Guinea-pig 278 inoculated with pus from the gland of Calf 16.

6. Testicles of Guinea-pig 285 inoculated with culture obtained from the pus from the gland of Calf 16.
7. Testicles of Guinea-pig 1 inoculated with culture from the testicles of Guinea-pig 275 (*q.v. supra*).
8. Local lesions of Calves 14, 21, and 22.

Cultural, morphological and staining characters.

Primary cultures, whether from natural or experimental lesions, as a rule grow slowly and are scanty. This is in agreement with the fact that it is generally impossible to discover the bacilli by means of the microscope in the pus, and with the escape from infection of a proportion of the animals inoculated.

Serum glycerine broth appears to be the best medium for primary cultures. It is a little difficult to determine exactly when there is a visible amount of growth in tubes of this medium because all the growth takes place at the bottom and its presence is masked by the pus used as seed material. This is more particularly the case because the pus cells appear to disintegrate in the broth and produce a flocculent deposit.

Microscopic examination establishes the fact in most cases that there is a considerable multiplication of the bacilli by the third or fourth day.

In the earlier experiments the absence of any visible growth on ordinary agar by the fourth day led to the abandonment of this medium for primary cultures. In the second series of experiments serum glycerine agar was found to be a very suitable medium for primary cultures from the lesions of experimentally infected animals.

Subcultures in serum broth or serum glycerine broth shew after 24 hours incubation a small amount of white flocculent deposit at the bottom of the tubes only. The broth remains quite clear. It requires some amount of agitation to disturb the sediment, and even vigorous shaking is not sufficient to break up the flocculi completely. In from one-third to one-half the tubes inoculated a surface scum begins to form about the third day. This scum is uneven in thickness and in the thicker parts has a faint buff tint. It is moderately coherent, since if the tube is inclined and then rotated the scum can be made to adhere to the glass without breaking up. When the rotation of the tube is continued it returns to its position on the surface of the broth. Comparatively slight agitation will cause it to sink.

In primary cultures on serum agar or serum glycerine agar the organism develops small round translucent colonies which are colourless by transmitted light and have a slight whitish tint by reflected light.

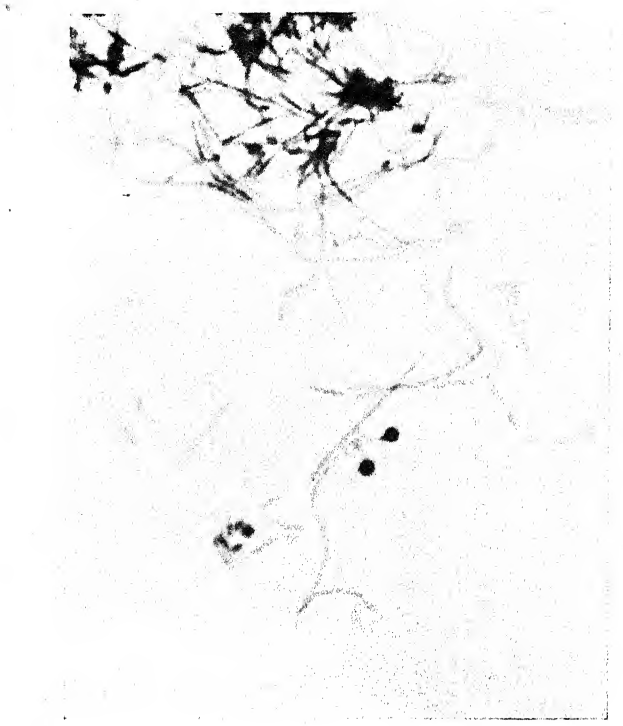


Fig. 1. Serum broth culture, 3rd, testicle, Guinea-pig 252. Bottom growth at 24 hours ×2000.

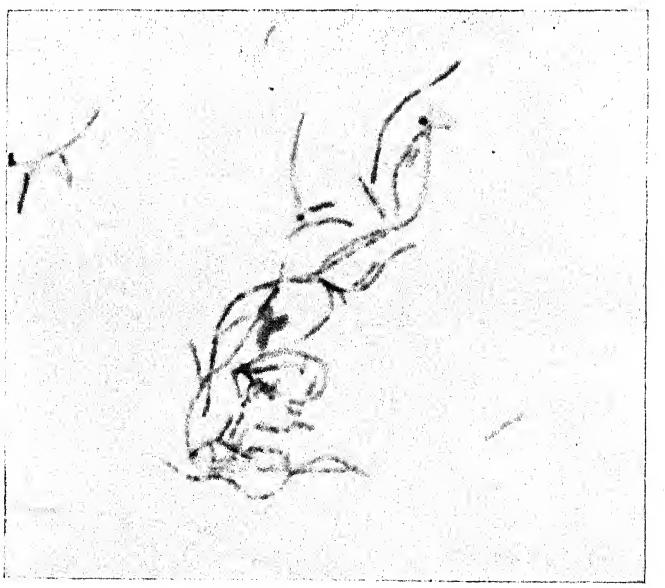


Fig. 2. Serum broth culture, 3rd, testicle, Guinea-pig 252. Bottom growth at 24 hours ×2000.

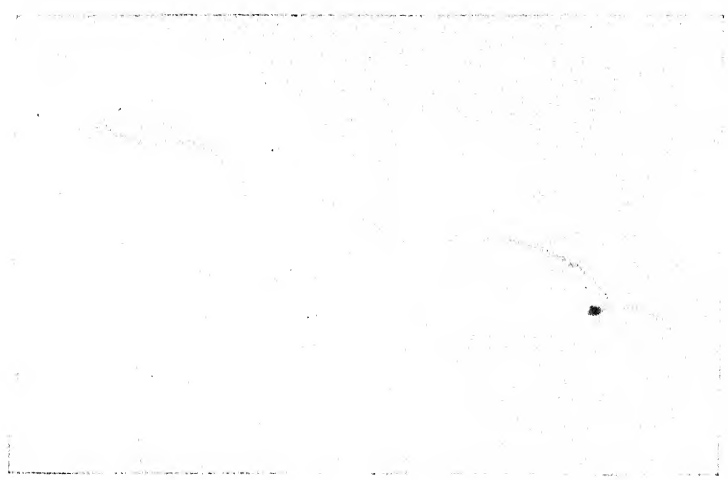


Fig. 1. Filament from serum broth culture, 3rd, testicle, Guinea-pig 252. Bottom growth at 24 hours ×2000.

Fig. 2. Serum broth culture, 3rd, testicle, Guinea-pig 252. Bottom growth at 24 hours ×2000.

These colonies are generally invisible to the naked eye until after the second 24-hours, but in some cases visible growths have been obtained from experimentally infected animals in 24 hours.

The growth does not shew any special characters to the naked eye, nor does microscopic examination of the individual colonies reveal any special details of structure or form.

In subcultures the growth takes the form of a continuous layer which is slightly yellowish by transmitted light and white by reflected light.

On neutral red agar the growth is like that obtained on serum agar but it acquires a strawberry pink colour.

No growth is obtained on potato.

Milk is not coagulated.

On a number of occasions cultures in Durham's liquid were tested for the presence of indol, but positive reactions were never obtained.

The bacillus can be stained by any of the simple aniline dyes, but it is not " fast " by the methods of Gram, Gram-Weigert, or Claudius.

A very good stain both for cultures and for specimens of pus was prepared on the same lines as Neisser's methylene blue, as originally prepared for the staining of the diphtheria bacillus, except that the methylene blue was replaced by basic fuchsin. Preparations stained with this liquid require no decolorization with acetic acid.

Good preparations were obtained with dilute carbol fuchsin and carbol gentian violet followed by 1 per cent. acetic acid. The acid could be left acting for a minute or more without appreciably decolorizing the bacilli.

Morphology of the organism.

A. In lesions. As already mentioned the bacillus is extremely difficult to detect microscopically in pus from natural lesions, but in the few cases in which it was found it occurred in the form of a small bacillus either singly or in small groups. The bacilli measured from 2 to 5 microns in length and the thickness varied from 0·3 to 0·6 microns. (Plate II, fig. 2.)

The majority of them did not stain evenly. Some shewed a stained speck at each end with a colourless central part, while others presented a distinctly beaded appearance.

B. In cultures. The morphology of the bacillus in culture varies with the nature of the medium and the age of the culture.

Growth taken from the sediment of a 24-hour broth culture is mostly in the form of long filaments which are intricately twisted and coiled together,

These filaments are not all alike in appearance, some are stained somewhat faintly and shew just a suspicion of a beaded appearance. In others the beading is quite distinct. In still others there is clear segmentation into bacilli.

Even after 24-hour incubation what appear to be involution forms are discoverable. Some of the filaments have rounded swollen ends and some of the bacilli are large and spindle-shaped. (Plates III and IV.)

A portion of the culture is composed of separate bacilli resembling those found in the pus and these shew the same variation in size and staining reactions.

Preparations made from the surface growth of a 24-hour serum agar or serum glycerine agar culture shew that the bulk of the growth is composed of separate bacilli. A small number of filaments are usually discoverable, but these are not more than 30 to 40 microns in length. The individual bacilli vary considerably in size. Many bear a close resemblance to the bacillus of fowl cholera, while others are very like the bacillus of glanders. Granular as distinct from beaded forms may be found in these preparations. They probably represent involution forms. (Plate V, fig. 1.)

After further incubation changes in the morphology of the bacillus become very marked. In preparations made from the bottom growth of a serum broth culture after incubation for 120 hours the regular filaments have practically disappeared. The growth is almost entirely composed of bacilli shewing great variation in shape and size, and all or nearly all less intensely stained than before. The illustration (Plate V, fig. 2) gives a better idea of the morphology of the bacillus at this stage than any written description can do.

Microscopic examination of the surface growth on serum broth during the first 24 hours after its appearance shows the same types of organisms as are present in the sediment after 24 hours, but it would appear that the segmentation of the filaments in the surface scum takes place somewhat more rapidly than in the bottom growth as they do not predominate to the same extent.

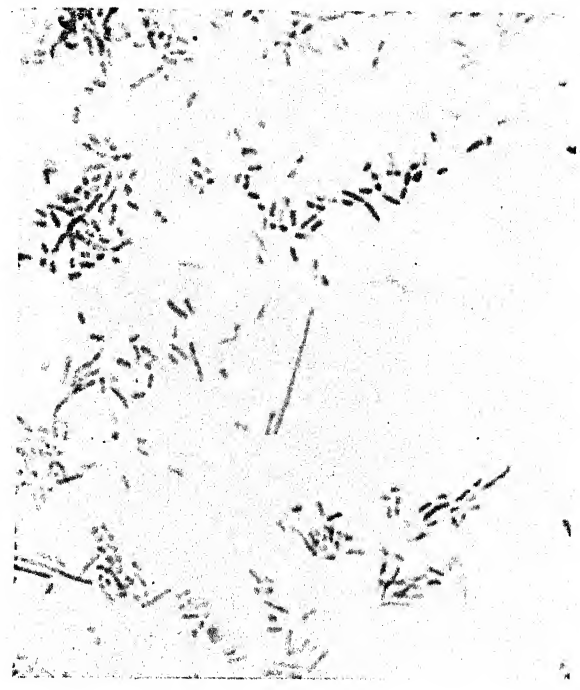


Fig. 1. Serum glycerine agar culture, 3rd, testicle, Guinea-pig 252. Bottom growth at 24 hours × 2000.

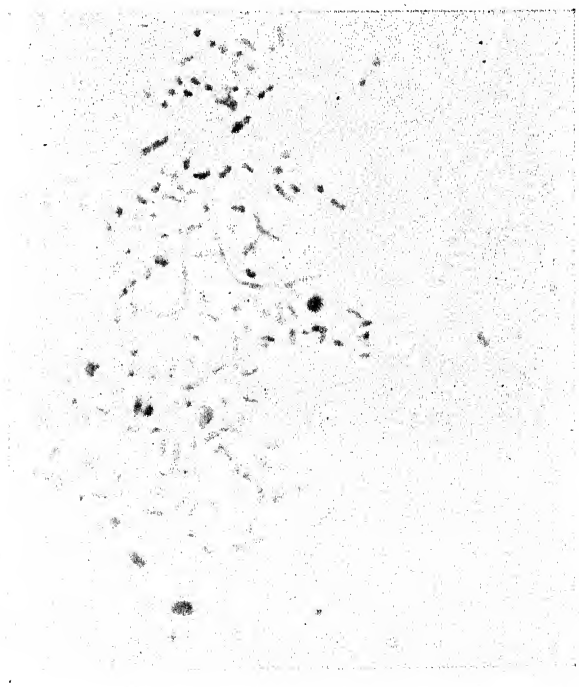
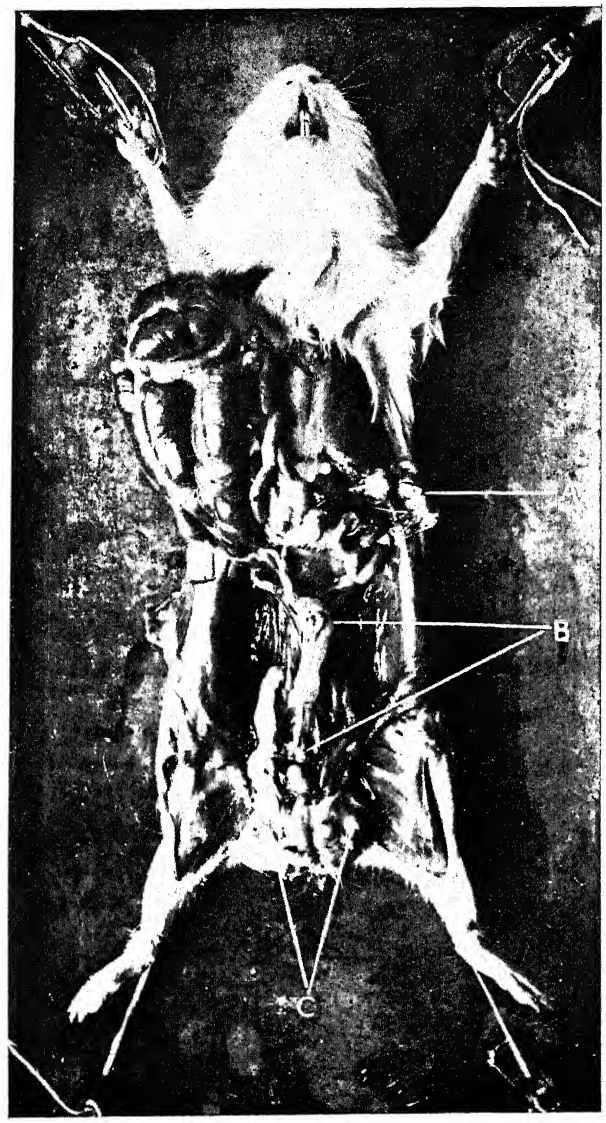


Fig. 2. Serum broth cultur , 3rd, testicle, Guinea-pig 252. Bottom growth after 120 hours' incubation × 2000.



The lesions produced by the intraperitoneal inoculation of Guinea-pig 46 with 0·5 c.c. of a suspension of the 3rd subculture from the testicles of Guinea-pig 252 on serum glycerine agar incubated 18 hours. Swelling of the testicles was appreciable on the 5th day and the animal was killed on the 16th day after inoculation.

At **A** there are five encapsuled abscesses in the omentum. The two lesions marked **B** are similar abscesses apparently developed on the course of the lymphatics of vesiculæ seminales. The lower of these lesions is also adherent to the upper surface of the bladder. At **C** are the two halves of the scrotum laid open shewing the complete conversion of the testicles into pus. Pus from the right half of the scrotum has run forward into the abdomen.

This guinea-pig is not referred to in the text. The inoculation was done after the manuscript had been completed.

SUMMARY.

With pus taken from natural cases of bovine lymphangitis it has been found possible to reproduce the disease in plains calves to the extent that subcutaneous inoculation has led to the formation of an abscess and suppuration of the nearest lymphatic gland. The condition so produced resembled the natural disease in that the lesion developed very slowly and the suppurating glands shewed little tendency to burst spontaneously; there was no rise of temperature and the animals shewed no general disturbance of health.

The intraperitoneal inoculation of male guinea-pigs with original pus has been followed in a very large proportion of cases by suppurative orchitis.

With cultures of an organism isolated from pus derived from animals experimentally infected with original pus it has been found possible to again produce similar lesions in both plains calves and guinea-pigs.

From the literature dealing with bovine lymphangitis it would appear that there are two distinct forms of the disease, one caused by a streptothrix (Nocard) and the other by a bacillus (Vryburg and Raymond).

After considering the reports of Raymond and Holmes regarding their investigations into the same outbreak, the only conclusion that one can come to is that Raymond's is by far the more acceptable.

The organism described in the present paper agrees in the majority of its characters with those described by Vryburg and Raymond. It differs in certain minor respects.

Raymond's organism is described as being negative to Gram's method of staining but positive to Gram-Weigert; the organism here described is negative to both. Vryburg does not mention the reaction to Gram-Weigert but his bacillus was negative to Gram.

Raymond's organism developed no surface growth on broth.

Both Vryburg and Raymond obtained positive indol reactions. None could be obtained with the bacillus here described.

Considering these differences it may be pointed out that there is sometimes room for a divergence of opinion as to whether an organism is or is not positive to Gram and its modifications. The bacillus described in this paper

was certainly negative to both as an exposure of ten seconds to the respective decolorizing agents was sufficient to render it almost invisible.

The development of a surface scum upon a proportion of the broth tubes was at first thought to be due to the presence of an impurity, but repeated plate cultivation indicated that it was not. A parallel case in which the development of a surface scum is not constant is to be found in the glanders bacillus.

The bacillus does not appear to agree in all its characters with any of the pus-producing bacilli hitherto described. It approximates most closely to the Preisz-Nocard bacillus but differs from that organism in being Gram-negative.

CONTENTS

STUDIES IN RINDERPEST.

BY

W. A. POOL, M.R.C.V.S.,

Officiating Director and First Bacteriologist, Imperial Bacteriological Laboratory, Muktesar,

AND

T. M. DOYLE, F.R.C.V.S.,

Veterinary Officer, Imperial Bacteriological Laboratory, Muktesar.

(Received for publication on 7th November, 1921.)

A. THE 'SERUM ALONE' METHOD OF INOCULATION FOR THE CONTROL OF RINDERPEST.

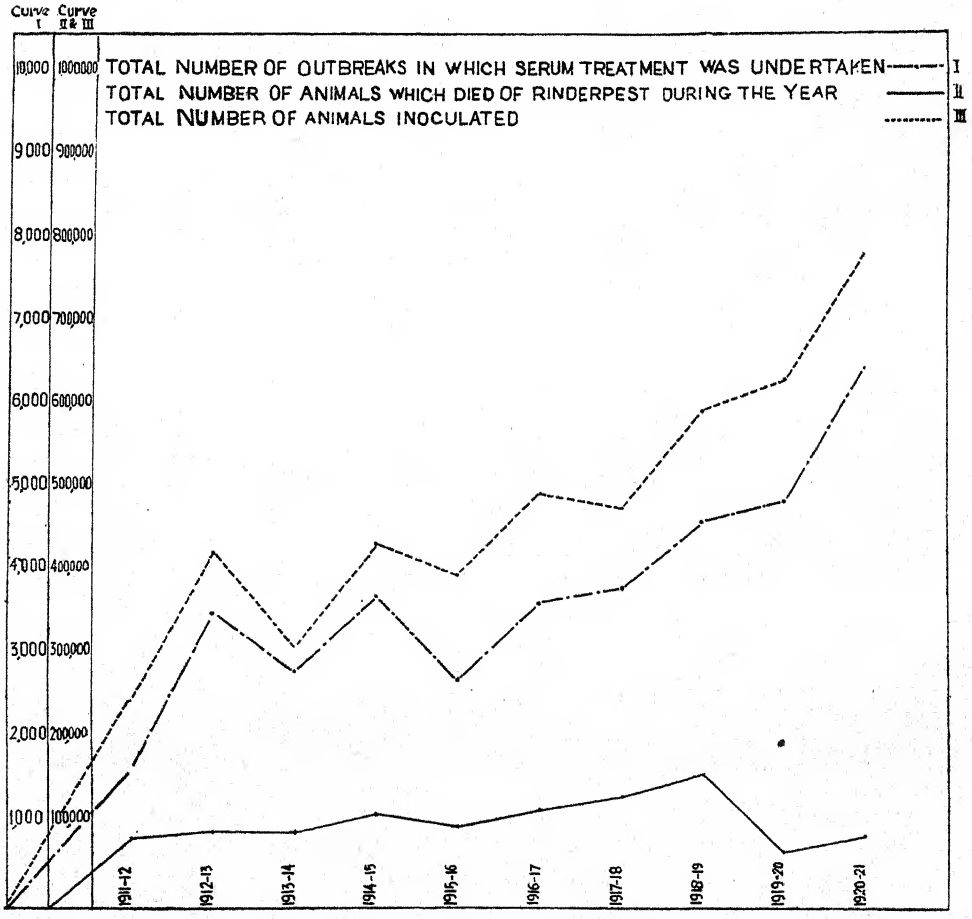
I. A survey of the results obtained in controlling rinderpest in India by the 'serum alone' method of inoculation.

THE 'serum alone' method of inoculation for the control of rinderpest was introduced into India more than twenty years ago as a result of the experience gained in dealing with the disease in South Africa at the close of the last century.

When it was first tried in this country, success was retarded by the opposition which cattle owners raised. This opposition was largely due to mistrust and partly due to apathy and religious principle though the latter was always given as the excuse. However, tactful and patient handling of the question by the Civil Veterinary Department gradually popularized the method, and at the present day it is in general use throughout India.

The present position has only been achieved after a great deal of propaganda work which among other things has involved consultation with the religious leaders and wide circulation of their opinions on the subject of inoculation as derived from an intimate knowledge of their holy scriptures.

Live-stock owners are conservative throughout the world and generally regard any innovation with suspicion which involves such a procedure as the inoculation of their herds through the agency of a State department. Even in Western countries and the New World, fresh measures for the control of epizootic diseases are often received by the people most intimately concerned with anything but enthusiasm.



In India the general low standard of education of the stock-owning classes accentuates this difficulty. In consequence the introduction of voluntary

inoculation carried out by Government veterinary officers was at first very difficult even though it was performed free of cost. The work was and is further hampered by the utterly inadequate veterinary staff available to deal with outbreaks of the disease.

There is no legislation to deal with cattle diseases, and inoculations can only be carried out with the full consent of the owners. All this was pointed out by Holmes[1], and he then considered that the serum alone method was the safest form of inoculation for general use in India.

It has served its purpose but was adopted as the best means of dealing with rinderpest when the very special conditions of Indian Administration had to be taken into consideration, rather than the best means of dealing with the disease itself.

It has been a safe way of dealing with uninfected animals and when carried out according to the teachings of Danysz and Bordet[2] and Holmes[1] there is a good chance of the inoculated animals acquiring an active immunity by contracting the infection from other animals suffering from the disease, while the passive immunity conferred by the serum is still existent.

There are, however, some pitfalls which at first were not very apparent The first of these is the duration of the immunity that the serum was believed to confer. As shown below, the duration of this immunity was over-estimated. Another important factor is the amount of infection that is present in an infected herd. Holmes' experiments were carried out on Kumaun Hill cattle which possess an extraordinarily high susceptibility to the disease. As shown later, the conditions in cattle of low susceptibility are somewhat different.

The above figure shows an important set of statistics in the form of a graph and from these it is apparent that the serum alone method of inoculation is a long way short of efficient for the control of rinderpest.

The reported mortality from the disease is enormous even though the number of outbreaks attended and the number of animals inoculated is going up in leaps and bounds.

It must be remembered that the actual mortality from the disease is almost certainly greatly in excess of that reported.

II. The duration of the immunity conferred by serum alone.

(*a*) The wide divergence in the views held as to the duration of the immunity.

Widely divergent opinions have been expressed by different investigators on the duration of the immunity conferred by rinderpest serum, and even at

the present day field workers generally appear to be very hazy on this fundamentally important point.

There is every excuse for this as laboratories issuing serum have themselves considerably over-estimated the period.

Up to the time of submitting this article for publication the circular giving directions for the use of anti-rinderpest serum issued by the Muktesar Laboratory stated that the immunity conferred by a single dose of serum is of short duration varying from 3 to 6 weeks and other laboratories state it to be about a month. Spreull[4] in 1897 in a communication to Hutcheon, Chief Veterinary Officer, Cape Colony, pointed out that the period of protection was about ten days.

Stockman concluded from his experience that serum alone gives an immunity for ten days only. He also stated[4] "as the best method of cutting short an outbreak and preventing the further spread, its adoption is only justifiable if it be used in such a manner as will ensure absolute immunity to the 'in contact' animals for a time equal to the duration of the infection." This still remains the soundest rule to be followed even though subsequent work was considered to point to rather different conclusions.

Nicolle and Adil Bey[5] in Turkey, Ruediger[6] and Jobling[7] in the Philippines and Head[8] in the Anglo-Egyptian Sudan have estimated the duration of the immunity at varying periods of from three weeks to three months.

Holmes reviewed the investigations which had been made with regard to this question to date (1909) and described some experiments of his own which, however, did not go far enough and left the question only half settled. He stated[1] :—

> "A single dose of anti-rinderpest serum confers immunity against the inoculated virus for about two weeks only."
>
> "A double dose protects for about three weeks, a treble dose for five weeks and four times the single dose for about six weeks."

When the experiments detailed below are considered it is obvious that with one or two exceptions the duration of the passive immunity conferred by serum alone has always been over-estimated and sometimes been grossly exaggerated. This factor combined with the use of an insufficient dosage for the susceptibility of the animals dealt with has led to a great deal of the disappointment which has been reported in the field.

(*b*) CONDITIONS WHICH MUST BE FULFILLED WHEN TESTING THE DURATION OF THE IMMUNITY CONFERRED BY SERUM ALONE.

A review of the literature shows that there has been a great lack of uniformity in fulfilling the requirements of experiments designed to elucidate problems in connection with this question. In consequence we now detail the requirements which we considered our own experiments should fulfil.

(*i*) *The potency of the serum must be accurately estimated.*

The *minimum* safe protective dose for the particular animals to be used in the experiments must be very accurately estimated. In the case of rinderpest serum there is always the difficulty that the only animals that can be used for test purposes are bovines, and the question of expense and of the available supply of highly susceptible animals generally prevents investigators from using as many in each test as is desirable. At the same time such a test has many advantages over those carried out on small laboratory animals as in the case of some other sera.

Holmes[1] states that he used serum which had been found to protect Kumaun Hill bulls at a dosage of 72 c.c. per 600 lb., but as pointed out on page 111 he does not say if this was the *minimum* protective dose.

Wood and Ward[9] carried out much experimental work in the Philippines on the value of serum immunity in dealing with rinderpest. The results of their experiments were so unfavourable that they advised that the use of serum should be discontinued and that methods of quarantine and disinfection entirely should be relied upon.

In the light of the experience gained in other parts of the world, such a conclusion would appear to be strange and must have been due to special conditions prevailing there.

A close examination of their experiments shows that an accurate estimation of the results was vitiated in each case by an insufficient knowledge of the potency of the serum they were using or in other words of the *minimum* dose of serum which was necessary to save the life of the animals with which they were experimenting.

They omitted to test the serum they used in some of their experiments and in others the tests carried out were insufficient.

It would appear that had larger doses of serum or more potent serum been used, they would have obtained very different results.

However they cleared up two very important points, *viz.*, that serum will not prevent infection but will only modify an attack of rinderpest. Also that the period of serum immunity is very short.

The same authors[1] in a later publication describe simultaneous inoculation in the field so they have probably modified these views.

On account of the very high output of serum from this Laboratory and of the ideal conditions that exist for rinderpest experimental work, a great deal of experience has been gained in testing serum before issue and in all the conditions which govern its use.

Our experience is that a highly potent serum gives uniform results in a test, in that it either protects all the animals under test in the ordinary dosage employed or gives a well-defined dividing line between the doses that protect and those that fail to protect. A very weak serum fails to protect any of the animals used and a serum of moderate potency does not produce uniform results.

Ward and Wood say "varying doses of serum did not produce sharp results." This corresponds with the results we have obtained when testing serum of *moderate* potency.

All the animals used for testing rinderpest serum at this Laboratory are Kumaun Hill bulls. They are ideal for the purpose as they show a ninety-eight per cent. mortality. Even in the case of animals with such a high susceptibility there is, however, some variation. Animals are not machines and biological tests which are dependent for their delicacy on individual animal characteristics must always be read with some latitude.

The apparently unsatisfactory results obtained when a serum of moderate potency is tested can easily be explained. If it is allowed that in animals showing a ninety-eight per cent. mortality there is a difference in individual susceptibility, and that even among the ninety-eight per cent. which die there is still an individual variation in this respect, it will be realized that a dose of serum which just saved the life of one of the animals may just fail to protect the next.

The routine test of a brew of rinderpest serum is described by Shilston[11] but the doses now used are 30 c.c., 60 c.c., and 90 c.c., respectively, per 600 lb. body weight.

When a moderate brew is tested, the 30 c.c. dose will probably prove insufficient even for the least susceptible animals of the class, both of the animals so inoculated dying from rinderpest. The two which received the medium dose may be the most resistant animals and may recover, while one of

the animals receiving the high dose may be the most susceptible (of the class) and die from the disease while the other recovers.

Such a result may at first sight appear to be very misleading but with a moderate brew an even greater anomaly may be shown such as one animal surviving and one dying with each dose, but it simply means that the serum is rather weak and is not sufficiently potent to overcome individual variation in susceptibility in the dosage used.

If the minimum protective dose for the animals under experiment is not known, many pitfalls arise. We have shown below that when an increase is given beyond the minimum for a single dose, the period of serum immunity conferred tends to be lengthened.

If the dosage used is insufficient the animals will receive no protection, or if it is nearly sufficient, a lack of uniformity will be obtained as reported by Ward and Wood[9].

In the serum under report most elaborate testing was resorted to. Six simultaneous tests were carried out with each brew (Tables I and III). As far as we can ascertain, these brews therefore have been put to more searching tests than any others which have previously been recorded.

(*ii*) *The methods of inoculating the virus and serum must be uniform.*

This should in both cases be by the subcutaneous route.

With such a short period of immunity (p. 15) there is always the possibility, when other methods are employed, that the infection may be contracted after the immunity conferred by the serum has started to diminish.

(*iii*) *The rinderpest virus used must be reliable.*

We consider that citrated or defibrinated virulent blood should always be used and that it should be taken from the virus producer while at the height of the disease and before the temperature falls as shown by Shilston[12].

Details of the reactions of Kumaun Hill bulls are given by Pool and Doyle.[13] We have no experience of the use of blood obtained from animals of moderate susceptibility for test purposes but have found[13] that virus obtained from such animals produces good serum.

Other methods of infection such as leaving the test animals in contact with infected animals or infected material are not so reliable, as one can never be certain when the disease was contracted. When the subcutaneous inoculation of virulent blood is so reliable we cannot see the advantage of

employing any other method. Other blood-borne diseases are of course set up by direct inoculation but we consider that when due care is taken, the absolute reliability of this method justifies its use.

In this connection Todd and White[14] say:—"At the Serum Institute, where animals were being daily inoculated with cattle plague for the production of virulent blood, there was no lack of infective material, and the special tubs at which these infected animals watered were used for the experiment.

"In order to increase the chance of infection, a special tub was reserved for animals suffering from fever, five and six days after inoculation, *i.e.*, at a time when the nasal discharge is known to be highly infective.

"Two animals were isolated in a special stable and were taken to water daily at the above tub directly after the infected animals had been watered. At the expiration of six weeks, as these animals remained well, they were tested with virulent blood and found to be susceptible.

"In a further experiment made under similar conditions to the last, two animals were watered at the infected tub for over three weeks, and, although susceptible, did not contract the disease.

"When we consider that in the last two experiments the susceptible animals were watered twice a day for several weeks at a tub where, less than half an hour before, an infected animal had drunk, and did not contract the disease, it is difficult to imagine that under natural conditions drinking water can play any important part in the spread of the infection."

The above of course only refers to infected drinking water but we consider that it bears out our own experience and helps to justify the conclusion drawn above.

(*iv*) *The test animals must be highly susceptible.*

For the production of really accurate results, it is essential that the animals used should be highly susceptible to the disease. When animals of low susceptibility are used, individual variation is far greater and the interpretation of results is very difficult. In many places highly susceptible animals are not available and in such cases an increase in the number of animals used is the only alternative. This is a very poor substitute as in any individual case it would not be possible to say if a negative result were due to lack of susceptibility of the individual or to passive immunity conferred by the serum.

The method adopted in India for the routine testing of the serum is ideal. It is tested on animals of very high susceptibility but the bulk of it is used for the inoculation of animals of low susceptibility. The serum issued is of

a reasonably level and certainly high potency and District Officers can learn by experience the dosage necessary for the cattle of their charge.

(*v*) *The animals must be free from other rinderpest infection while under test.*

This is of course an essential factor and when other rinderpest work is in progress at the same time, it is often a difficult condition to fulfil. The layout of the Muktesar Laboratory fortunately renders this factor a reasonably easy one to cope with, though cases have occurred in which an experiment has been nullified by the introduction of accidental infection.

This difficulty has been recognized and pointed out by other investigators and is a great source of delay in rinderpest research.

The following remarks by Todd and White [14] who were working in Egypt are interesting in this connection :—

" Experiments on the etiology and means of transmission of cattle plague present certain difficulties due to the conditions under which these experiments must necessarily be carried out. Owing to the highly infectious nature of the disease it is not possible to carry on many experiments at the same time ; as there must always be a considerable risk of accidental infection if any large number of infected and non-infected animals are brought into the same experimental areas, unless special precautions are taken. The unavoidable handling of the animals, necessitated by the taking of temperatures, etc., affords ample facility for the occurrence of cross infection ; especially in a country like Egypt, where the attendants are more or less untrained *fellahîn*. The impossibility of doing more than one or two experiments simultaneously, considerably retards the progress of such experimental work."

(*c*) A REVIEW OF THE EXPERIMENTS HOLMES CARRIED OUT TO ASCERTAIN THE DURATION OF THE IMMUNITY CONFERRED BY SERUM ALONE.

The test animals received a subcutaneous inoculation of virulent blood at varying periods after the injection of the serum.

Holmes[1] states : " The dose of this serum sufficient to protect against a simultaneous inoculation of virulent blood was fixed at 72 c.c. per 600 lb. body weight."

He does not give any details of the tests of the serum and in the above statement does not emphasize that this dose is the minimum required to protect. If, as is quite possible, this dose of the brew of serum used was a liberal one, a nda little more than the minimum required to confer complete protection , the result is that all the doses given were a little more than those stated, *viz.*, single dose, double dose, etc.

In that case the results of his experiments are not absolutely comparable with those we detail below.

Details of the experiments (Holmes).

(*i*) *Animals inoculated with a single dose of serum.* Three animals were tested after twenty-one days and three after twenty-four days and all died from rinderpest. Nine animals were tested after an interval of fourteen days. Five lived and four died.

(*ii*) *Animals inoculated with a double dose of serum.* Six animals were tested after twenty-eight days and three died and three survived. Three were tested after twenty-one days and all survived.

(*iii*) *Animals inoculated with a treble dose of serum.* Six animals were tested after an interval of thirty-eight days. Two died and four lived. Three animals were tested after an interval of twenty-eight days. Two died and one lived.

(*iv*) *Animals inoculated with five times the standard dose.* Three animals were tested after forty-four days and all lived.

From these results the following conclusions can be drawn but they differ somewhat from those drawn by Holmes :—

(*i*) A single dose of serum did not confer complete protection for fourteen days.

(*ii*) A double dose of serum conferred immunity for twenty-one days.

(*iii*) A treble dose of serum did not confer immunity for twenty-eight days.

(*iv*) Five times the standard dose of serum conferred immunity for forty-four days.

(*d*) DETAILS OF SOME EXPERIMENTS DESIGNED TO ASCERTAIN MORE ACCURATELY THE DURATION OF SERUM ALONE IMMUNITY.

The intervals after which the animals were tested were adjusted after reviewing the literature on the subject quoted above. It was obvious that the serum immunity did not last for fourteen days and it was deemed advisable to settle accurately the maximum duration conferred with certainty by a single dose as this is the deciding factor in controlling the disease in the field by means of serum alone.

Experiment No. 1.

Brew A of serum which was used for these experiments was subjected to six simultaneous tests (Table I).

TABLE I.

A summary of six simultaneous tests of serum brew A used in Experiment No. I.

The test animals and controls were inoculated with 0·5 c.c. of virulent blood from hill bull No. 565, temperature 104·4°F., vesicles 5th day of attack.

Number of hill bulls used	Dose of serum per 600 lb. body weight c.c.	Virus c.c.	Recovered	Died
12	30	0·5	9	3
12	60	0·5	9	3
12	90	0·5	12	nil

Controls.

Hill bull No. 395, temperature 104·0°F., vesicles 6th day of attack.
Hill bull No. 622, temperature 104·2°F., vesicles 5th day of attack.

Conclusion.

The protective dose of serum brew A is 90 c.c. per 600 lb. for hill cattle.

TABLE II.

Experiment No. I.

A

Length of immunity conferred by a *single* dose of serum.

Test animals and controls inoculated with 0·5 c.c. of virulent blood from-

Hill bull No. 391, temperature 104·0°F, vesicles 5th day of attack.
Hill bull No. 571, temperature 104·4°F., vesicles 6th day of attack.

Number of test animal hill bull	Body weight lb.	Actual dose of serum c.c.	Test after interval of days	Virus c.c.	Virus from hill bull No.	Maximum temp. °F.	Vesicles. Day	Result
533	190	29	10	0·5	391	103·8	8th	Died 10th day.
532	217	32	10	0·5	391	104·8	8th	Moderate reaction. Recovered.
531	270	40	10	0·5	391	104·2	8th	Moderate reaction. Recovered.
534	297	45	14	0·5	571	104·3	5th	Died 9th day.
535	179	27	14	0·5	571	104·6	6th	Died 8th day.
536	170	25	14	0·5	571	104·8	8th	Died 11th day.
Controls	Inoculated from H.B. 391. Hill bull No. 394, temperature 104·6°F., vesicles 5th day.							
	,, ,, ,, ,, Hill bull No. 573, ,, 104·5°F., vesicles 6th day.							
	,, ,, ,, 571. Hill bull No. 662, ,, 104·0°F., vesicles 5th day.							

TABLE II.—(*contd.*)

B

Length of immunity conferred by a *double* dose of serum.

Test animals and controls inoculated with 0·5 c.c. of virulent blood from—

Hill bull No. 396, temperature 104·4°F., vesicles 5th day of attack.

Hill bull No. 733, temperature 104·2°F., vesicles 4th day of attack.

Number of test animal hill bull	Body weight lb.	Actual dose of serum c. c.	Test after interval of days	Virus c.c.	Virus from hill bull No.	Maximum temp. °F.	Vesicles. Day	Result
537	243	73	18	0·5	396	104·5	7th	Died 10th day.
538	194	58	18	0·5	396	105·5	8th	Moderate reaction. Recovered.
539	193	58	18	0·5	396	104·4	9th	Moderate reaction. Recovered.
541	165	49	21	0·5	773	104·5	8th	Moderate reaction. Recovered.
623	350	105	21	0·5	773	104·6	8th	Severe reaction. Recovered.
624	360	108	21	0·5	773	104·2	6th	Died 15th day.

Controls	Inoculated from H.B. 396.	Hill bull No. 672, temperature 104·0°F., vesicles 4th day.
	„ „ „ „	Hill bull No. 687, temperature 104·2°F., vesicles 5th day.
	„ „ „ 733.	Hill bull No. 759, temperature 104·6°F., vesicles 5th day.

C

Length of immunity conferred by a *treble* dose of serum.

Test animals and controls inoculated with 0·5 c.c. of virulent blood from—

Hill bull No. 711, temperature 104·4°F., vesicles 5th day of attack.

Hill bull No. 727, temperature 104·2°F., visicles 5th day of attack.

Number of test animal hill bull	Body weight lb.	Actual dose of serum c. c.	Test after interval of days	Virus. c.c	Virus from hill bull No.	Maximum temp. °F.	Vesicles. Day	Result
545	213	140	32	0·5	711	104·0	7th	Died 9th day.
544	270	122	32	0·5	711	105·4	6th	Died 12th day.
543	427	192	32	0·5	711	104·6	6th	Died 15th day.
546	359	162	35	0·5	727	103·8	6th	Died 8th day.
547	130	59	35	0·5	727	103·6	6th	Died 9th day.
548	355	160	35	0·5	727	104·6	8th	Died 11th day.

Controls	Inoculated from Hill Bull 711.	Hill bull No. 729, temperature 104·8°F., vesicles 6th day.
	„ „ „ „ „	Hill bull No. 733, temperature 104·4°F., vesicles 5th day.
	„ „ „ „ 727.	Hill bull No. 749, temperature 104·2°F., vesicles 5th day.
	„ „ „ „ „	Hill bull No. 750, temperature 104·6°F., vesicles 5th day.

TABLE II.—(*concld.*)

D

Length of immunity conferred by *four times* the normal dose of serum.
Test animals and controls inoculated with 0·5 c.c. of virulent blood from—
Hill bull No. 766, temperature 104·6°F., vesicles 6th day of attack.
Hill bull No. 754, temperature 104·2°F., vesicles 5th day of attack.

Number of test animal hill bull	Body weight lb.	Actual dose of serum c.c.	Test after interval of days	Virus c.c	Virus from hill bull No.	Maximum temp. °F.	Vesicles. Day	Result
549	231	139	38	0·5	766	104·6	9th	Severe reaction. Recovered.
625	410	246	38	0·5	766	104·2	9th	Severe reaction. Recovered.
552	364	218	42	0·5	754	106·2	8th	Severe reaction Recovered.
554	385	231	42	0·5	754	105·2	9th	Died 12th day.

Controls	Inoculated	from H.	B.	766.	Hill bull No. 778, temperature 105·2°F., vesicles 5th day.
	„	„	„	„	Hill bull No. 777, temperature 105·6°F., vesicles 5th day.
	„	„	„	754.	Hill bull No. 763, temperature 104·6°F., vesicles 5th day.
	„	„	„	„	Hill bull No. 762, temperature 104·0°F., vesicles 5th day.

Conclusions.

1. A single dose of serum gave no protection after 14 days and did not give complete protection after 10 days.

2. A double dose of serum did not give complete protection after 18 days.

3. A treble dose of serum gave no protection after 32 days.

4. Four times the normal dose of serum gave protection after 38 days.

Experiment No. 2.

The intervals after which the animals were tested, were modified in view of the results obtained in Experiment No. 1. Brew B of serum which was used for this experiment was subjected to six simultaneous tests **(Table III).**

TABLE III.

A summary of six simultaneous tests of serum brew B used in Experiment No. 2.

The test animals and controls were inoculated with 0·5 c.c. of virulent blood from Hill bull No. 1314, temperature 104·0°F., vesicles 5th day of attack.

Number of hill bulls used	Dose of serum per 600 lb. body weight c. c.	Virus c.c.	Recovered	Died
12 ..	30	0·5	11	1
12 ..	60	0·5	12	nil
12 ..	90	0·5	12	nil

Controls.

Hill bull No. 1321, temperature 104·0°F., vesicles 5th day of attack.

Hill bull No. 1413, temperature 105·0°F., vesicles 5th day of attack.

Conclusion.

The protective dose of serum brew B is 60 c.c. per 600 lb. body weight for hill cattle.

TABLE IV.

A

Length of immunity conferred by a *single* dose of serum.

Test animals and controls were inoculated with 0·5 c.c. of virulent blood from—

Hill bull No. 1596, temperature 104·0°F., vesicles 5th day of attack.

Hill bull No. 1602, temperature 104·2°F., vesicles 7th day of attack.

Number of test animal hill bull	Body weight lb.	Actual dose of serum c.c.	Test after interval of days	Virus. c.c.	Virus from hill bull No.	Maximum temp. °F.	Vesicles. Day	Result
1617	93	9·3	9	0·5	1596	104·0	8th	Moderate reaction. Recovered.
1618	110	11·0	9	0·5	1596	105·0	8th	Moderate reaction. Recovered.
1619	380	38·0	9	0·5	1596	103·0	nil	Slight reaction. Recovered.
1614	129	12·9	12	0·5	1602	102·0	7th	Died 10th day.
1615	140	14·0	12	0·5	1602	103·6	6th	Died 9th day.
1616	117	11·7	12	0·5	1602	104·6	5th	Died 9th day

Controls { Inoculated from H. B. 1596. Hill bull No. 1607, temperature 104·2°F., vesicles 6th day.
" " " 1602. Hill bull No. 1611, temperature 104·0°F., vesicles 6th day.

TABLE IV.—(*contd.*)

B

Length of immunity conferred by a *double* dose of serum.

Test animals and controls inoculated with 0·5 c.c. of virulent blood from—

Hill bull No. 617, temperature 103·8°F., vesicles 5th day of attack.

Hill bull No. 1640, temperature 104·0°F., vesicles 6th day of attack.

Number of test animal hill bull	Body weight lb.	Actual dose of serum c. c.	Test after interval of days	Virus. c. c.	Virus from hill bull No.	Maximum temp. °F.	Vesicles. Day	Result
1634	204	40·8	16	0·5	617	102·8	nil	Slight reaction. Recovered.
1635	240	48·0	16	0·5	617	106·0	5th	Died 9th day.
1636	165	33·0	16	0·5	617	103·4	5th	Died 13th day.
1659	280	56·0	24	0·5	1640	105·0	7th	Severe reaction. Recovered.
1660	125	25·0	24	0·5	1640	104·8	7th	Died 11th day.
1661	107	21·4	24	0·5	1640	105·2	7th	Died 9th day.

Controls { Inoculated from H. B. 617. Hill bull No. 1633, temperature 104·0°F., vesicles 5th day.
„ „ „ 1640. Hill bull No. 1665, temperature 103·6°F., vesicles 6th day.

C

Length of immunity conferred by a *treble* dose of serum.

Test animals and controls inoculated with 0·5 c.c. of virulent blood from—

Hill bull No. 1666, temperature 104·4°F., vesicles 5th day.

Hill bull No. 1640 (see B).

Number of test animal hill bull	Body weight lb.	Actual dose of serum c.c.	Test after interval of days	Virus c.c.	Virus from hill bull No.	Maximum temp. °F.	Vesicles. Day	Result
1662	155	46·5	24	0·5	1640	103·4	7th	Died 11th day.
1663	114	34·2	24	0·5	1640	103·2	nil	No reaction.
1664	425	127·5	24	0·5	1640	103·6	nil	Slight reaction. Recovered.
1672	467	140·1	30	0·5	1666	103·0	9th	Moderate reaction. Recovered.
1673	331	99·9	30	0·5	1666	103·8	9th	Died 10th day.
1674	384	114·3	30	0·5	1666	104·4	8th	Died 10th day.

Control. Inoculated from H.B. 1666. Hill bull No. 1658, temperature 104·4°F., vesicles 5th day.

TABLE IV.—(*concld.*)

D

Length of immunity conferred by *four times* the normal dose of serum.

Test animals and controls inoculated with 0·5 c.c. of virulent blood from—

Hill bull No. 1668, temperature 105·2°F., vesicles 5th day.

Hill bull No. 1696, temperature 104·0°F., vesicles 6th day.

Number of test animal hill bull	Body weight lb.	Actual dose of serum c.c.	Test after interval of days	Virus c.c.	Virus from hill bull number	Maximum temp. °F.	Vesicles. Day	Result
1679	290	116·0	32	0·5	1668	103·6	8th	Died 11th day.
1680	355	142·0	32	0·5	1668	103·8	9th	Moderate reaction. Recovered.
1681	302	120·0	32	0·5	1668	103·4	7th	Died 8th day.
1704	240	96·0	40	0·5	1696	103·4	8th	Moderate reaction. Recovered.
1705	252	100·8	40	0·5	1696	104·2	6th	Died 9th day.
1706	203	81·2	40	0·5	1696	105·4	7th	Died 13th day.
Controls	Inoculated from H.B. 1668. Hill bull No. 1702, temperature 104·6°F., vesicles 5th day.							
	„ „ „ 1696. Hill bull No. 1710, temperature 105·0°F., vesicles 6th day.							

E

Length of immunity conferred by *ten times* the normal dose of serum.

Virulent blood and controls as in D.

Number of test animal hill bull	Body weight lb.	Actual dose of serum c.c.	Test after interval of days	Virus c.c.	Virus from hill bull No.	Maximum temp. °F.	Vesicles. Day	Result
1711	268	268	42	0·5	1696	105·0	9th	Died 13th day.
1712	183	183	42	0·5	1696	104·6	7th	Severe reaction. Recovered.
1713	185	185	42	0·5	1696	105·0	7th	Severe reaction. Recovered.

Conclusions.

1. A single dose of serum conferred complete protection for nine days.
2. A double dose of serum failed to protect for sixteen days.
3. A treble dose of serum did not give complete protection for twenty-four days.

4. Four times the normal dose of serum failed to protect for thirty-two days.

(*e*) General conclusions.

1. A single dose of anti-rinderpest serum confers a passive immunity for nine days.

2. Increased doses of serum prolong the immunity but not in proportion to the increase.

From the above it is apparent that it will be profitable to prosecute the enquiry further and to ascertain the maximum period for which a double dose, etc., will confer immunity and the maximum period of immunity that can be conferred by any dose of serum or in other words the longest possible period of passive immunity that can be conferred by anti-rinderpest serum.

Further work in this direction is in progress.

The results of these experiments will be of interest in connection with other sera, both human and veterinary.

III. The nature of the immunity conferred by anti-rinderpest serum.

This point was discussed by Ward and Wood[7] who came to the conclusion that the injection of the serum does not prevent infection with rinderpest.

They say: "On the contrary animals injected with serum and exposed to rinderpest soon contract the disease and pass through a more or less modified attack. We have shown that the blood of animals is infective during this attack. If by passive immunity is meant an artificial condition by means of which the severity of an attack is lessened, we grant that such exists, but deny that there is a passive immunity of a kind which prevents invasion by the virus of rinderpest."

We have shown later that even when very large doses of serum are used in the serum simultaneous method of inoculation, immunity to rinderpest is acquired as proved by failure to react to an infection of virulent blood at a later date when all possibility of passive protection by the serum has worn off.

This result corroborates the conclusions of Ward and Wood quoted above.

IV. Conditions which influence the efficiency or otherwise of the serum alone method of inoculation.

It is in countries in which the system of administration does not allow the employment of strict sanitary measures that rinderpest is a permanent problem at the present day, and in these countries it is the cattle of moderate

or low susceptibility that keep the disease smouldering so that a spark ignites whenever the conditions are favourable.

In a herd of cattle of low susceptibility such as most of the plains cattle of India, the spread of the disease is often very slow and the infection is probably kept alive by chronic bowel cases which are capable of spreading it for a period far in excess of the duration of the serum immunity.

As shown above, repeated inoculations every nine days until the disease disappears will remedy this, but apart from the question of expense, the inadequacy of the Veterinary Department in India does not allow of this procedure.

This means that when rinderpest is present, the local Veterinary Assistant who usually has charge of a very large area, probably receives reports of its existence from many places at once and that by the time he has done his round, in any individual instance there has often been very considerable delay between its onset in the village and the completion of the inoculations. This delay is at least a matter of days and sometimes a matter of weeks.

By the time the inoculations are performed the chances are that the most susceptible animals have either succumbed to the disease or already contracted it with the result that only the less susceptible animals are left.

The method employed in India of mixing the serum-inoculated cattle with naturally infected cases is theoretically an ideal measure but its defects are very obvious and numerous.

The degree of susceptibility of the different breeds of cattle has a great influence on the results obtained from the serum alone method. This is an important question and it has not always received the consideration which is necessary. When a herd of highly susceptible cattle is attacked, the infection runs through it in a very short time and either rinderpest or the hosts are soon stamped out.

When promptly carried out it gives better results in outbreaks amongst highly susceptible animals than among those possessing a partial resistance to the disease.

A recent outbreak of rinderpest among hill cattle in one of the outkraals of this Laboratory gave an opportunity of accurately estimating the percentage of animals which would contract an active immunity as the result of a very prompt injection of serum followed by the mixing of the healthy animals with cases of natural infection.

All the healthy animals received an injection of serum in the standard dose (90 c.c. per 600 lb.). Two months later they were taken in small batches to the rinderpest sheds for use as virus producers. Each animal received 5 c.c.

virulent blood and only five per cent. reacted. Therefore ninety-five per cent. had acquired an active immunity.

The mixing of the animals was unavoidable as at that time we had a very large stock and it was not possible to isolate them with the result that the conditions were as described above.

In this connection Ware[1] states :—" In dealing with outbreaks of rinderpest in villages great difficulty is found at least in some parts of the country, in persuading the people to give their cattle (inoculated with serum) the opportunity of contracting the disease. "

In such circumstances serum alone inoculation is really dangerous as it may lead to a sense of false security. The animals lose their serum protection in a few days and are then as susceptible as they were before. The infection is likely to be present for some time after their serum protection has worn off so that they can then contract rinderpest. In this way inoculation becomes unpopular and the progress of the department is retarded.

B. THE SERUM SIMULTANEOUS METHOD OF INOCULATION FOR THE CONTROL OF RINDERPEST.

I. A general consideration of the serum simultaneous method of inoculation.

This is the method *par excellence* for a country such as India where the disease is enzootic. The principle objection to it is that the inoculated cattle are given active rinderpest and are thus capable of infecting susceptible animals while they are reacting. When it is employed for stamping out the disease, the infectiousness of the animals inoculated is of no importance as it is already existent. In countries where it is wide-spread and animals are constantly exposed to the infection, it is the most reliable and economical of all methods.

In the directions for carrying out the inoculation, Pool[16] was considering herds which consist largely of valuable stock with a large proportion of imported blood and advising a policy which would err on the side of safety.

As soon as sufficient experience has been gained in the districts if the method is adopted generally in India, it is likely that a single visit will prove sufficient for inoculating the cattle and say a subsequent one about a week later to see how they are progressing and to treat any showing piroplasmosis or other disease.

A great advantage is that more outbreaks of the disease can be attended by the same staff than is possible when the serum alone method is employed.

The economical side of the question was realized by the first meeting of Veterinary Officers in India[17] who after fully discussing it from all sides and taking into consideration conditions in India, including religious prejudice, passed the following resolution unanimously : " (Resolution 12) That when an adequate and sufficiently trained staff is available, the question of adopting

the simultaneous method of inoculation against rinderpest more generally might be taken up as being more economical and effective."

When the efficiency of this method is contrasted with the poor results obtained by a single visit for serum alone inoculation or the expense in the cost of salaries, serum and other expenses of the staff required for repeated visits every nine days until the disease has been stamped out, there can be no question that the weight of evidence is all in its favour. One of the chief difficulties in the employment of the method is the production of suitable virus but we consider that it is possible to meet this contingency.

The greatest care must be taken in the selection of virus producers as this is the source from which all the mortality arises. The chief cause of mortality is piroplasmosis and so far it has not been found to be as dangerous in India as it is in some other countries.

We would here note that in our opinion bovine piroplasmosis in India requires further investigation. The different piroplasms have not been definitely identified in the light of recent researches in other countries ; therefore in this paper the term piroplasmosis is used throughout in its widest sense.

It is a well-known fact that the degree of virulence of piroplasmosis varies from one locality to another, and that an animal immune in one locality may contract a fatal infection if moved to another. Virus producers should therefore as far as possible be obtained from the same area as the cattle to be inoculated so that the risk of introducing a more virulent strain of piroplasmosis into the inoculated cattle may be avoided.

It must be remembered that the experience of the serum simultaneous method of inoculation in India outside Muktesar is practically limited to work done with the cattle of the military dairies and with the herd at the Agricultural Research Institute, Pusa. This has meant that the virulent blood used has been obtained from hill bulls at Muktesar and Bareilly or from virus producers derived from the herds in question. The hill bulls apparently do not harbour a very virulent strain of piroplasmosis and the latter are kept in very different conditions from village cattle but it is possible that when the method is extended to the districts, the virus producers used may pass on more virulent strains of protozoan infections than has hitherto been found to be the case.

In an unpublished report to the Government of Madras, Shilston stated : " The risk of inducing red-water in imported stock by the blood inoculation can be avoided when blood from a hill bull is used as virus, by storing this for eight days before injection, the organism of red-water will then have perished but the rinderpest virus will still be active.

"Blood from plains animals however does not usually remain virulent more than a few days, and so cannot be stored in this way."

The employment of virus producers of the same locality as the animals to be inoculated should minimize this danger to a large extent and at any rate apart from the above, it is the most that can be done with our present knowledge.

As an argument in favour of this method of controlling rinderpest, we will consider an imaginary outbreak in a herd of a hundred ordinary plains village cattle of moderate susceptibility and contrast the probable results which will follow when —

(*a*) No inoculations are performed and the disease is allowed to run its natural course.

(*b*) A single serum alone inoculation is carried out seven days after the first case of the disease is diagnosed.

(*c*) Serum simultaneous inoculation is carried out seven days after the first case of the disease is diagnosed.

We will assume that twenty of the animals will be of high susceptibility and will die in spite of treatment (short of serum inoculation prior to infection). We will call these group A.

Probably another thirty will be moderately susceptible and half of them will recover and half of them will die. We will call these group B.

Probably the remaining fifty will be of very low susceptibility and will recover in ordinary circumstances even if infected. We will call these group C. Thus the herd will show a thirty-five per cent. mortality if all become infected.

The hundred cattle belong to ten different owners and we will assume that each owner has ten cattle.

Before the onset of the disease the conditions under which the animals are kept such as the grazing and working, etc., entail a certain amount of intercommunication between the different batches.

After the disease appears the sick animals are each kept in their owners' yards and are nursed so that they do not go to a common grazing ground. At night the cattle of each owner are housed together in the same way as they were before the disease appeared so that the sick and healthy animals of any one owner are mixed.

The village *chamars* get the carcases of any animals that die. They skin them and generally it may be considered that each carcase is a source of infection for a short time afterwards.

The animals that recover are turned out to graze as soon as they are convalescent. It is likely that most of them cease to be infective very soon

after they are convalescent but a study of the epizootiology of rinderpest leads one to believe that there is a very strong possibility that some animals become 'carriers' and remain so for a considerable period.

Thus we may say that in this village the opportunities of the healthy animals picking up the infection are very haphazard; there is neither systematic mixing nor segregation but there is a certainty that the infection at least to a limited extent will hang about for months unless the disease is stamped out.

As a basis for argument the Chart opposite shows the course of the disease week by week under each of the three conditions. In it we have first worked out the possible incidence when no inoculations are carried out and then modified the conditions according to the average probabilities when each of the two methods of inoculation are carried out. We have assumed that the first case is diagnosed on the fourth day of the first week.

The Chart shows that when the outbreak was allowed to take its natural course, the disease ran on for thirteen weeks. Nineteen of group A became infected and none recovered. Twenty-five of group B became infected and thirteen recovered (52 per cent.). Twenty-five of group C became visibly infected and recovered. It may be assumed that at least another fifteen of this group would have shown no reaction if inoculated with virulent blood alone (without serum protection), that they had opportunities for acquiring infection and would have shown it if they had not been immune.

One animal of group A, five of group B and probably some of group C escaped the infection and were still susceptible to the disease.

When serum alone inoculation was carried out, seventeen of group A became infected and eight recovered (49 per cent.), twenty-two of group B became infected and thirteen recovered (59 per cent.) and twenty of group C became visibly infected.

Eight animals of group A and about four more of group B got infected while the serum immunity was serviceable and recovered, and about five more of group C probably acquired infection while under the protection of the serum and showed no reaction. Three animals of group A, eight of group B and some of group C escaped the infection and were still susceptible to the disease.

Thus the serum alone inoculation saved the life of about twelve animals which died when the disease ran its natural course. It speeded up the course of the disease owing to the extra opportunities which occurred for contracting the infection while the animals were collected for the inoculation. The infection was eliminated a week earlier than when it was allowed to run its

natural course but the inoculation absolutely failed to stamp it out. As the inoculation was carried out on the fourth day of the second week, practically all the serum immunity had worn off by the end of the third week. During the latter end of the third week and during the fourth week the infection remained and the animals that had not yet acquired the infection were as susceptible as ever.

The dose of serum used was of course sufficient to protect even the most susceptible animals.

When serum alone and serum simultaneous inoculations were carried out, the animals that had already acquired the infection behaved of course in the same way as when the disease was allowed to run its natural course.

When serum simultaneous inoculation was carried out, active immunity was conferred so that as the dose of serum used was sufficient to protect even the most susceptible animals, no more died from rinderpest. One animal of group C died from a complication, probably piroplasmosis, which gave a mortality from the inoculation of over one per cent.

To sum up, the following table contrasts the important points.

TABLE V.

	CATTLE UNINOCULATED				SERUM ALONE INOCULATION				SERUM SIMULTANEOUS INOCULATIONS			
	Groups			Total	Groups			Total	Groups			Total
	A	B	C		A	B	C		A	B	C	
Mortality	19	12	—	31	9	9	—	18	5	2	1	8
Duration of outbreak in weeks	13				12				5			

We submit that taking into consideration the imaginary circumstances in which the disease occurred, and dealing with each phase according to the natural probabilities, the above table is a fair estimate of the average results that would be expected in each case.

We have tried to assume fairly conditions which we have encountered in Indian villages and contend that the great advantages of the serum simultaneous method of inoculation over all other methods of dealing with the disease, where it is enzootic, are clearly demonstrated.

II. A survey of the results obtained by the serum simultaneous method of inoculation.

(*a*) In India.

The difficulty in introducing this method of inoculation into India for general district use has often indirectly been emphasized in the past in publications and correspondence from officers of the Indian Civil Veterinary Department, and until recently it has always been considered to be impractical. As stated above, the special conditions in India limit the powers of the Civil Veterinary Department for dealing with contagious disease, so that in the control of rinderpest, serum alone inoculation has always been given preference on account of its safety, because it was considered that the extra efficiency of serum simultaneous inoculation did not sufficiently compensate for the probable dangers it involves. As shown later, these dangers have been exaggerated.

A hindrance to its introduction has been the very wide variation in the susceptibility of Indian cattle and the further wide variation which is found when imported animals have to be dealt with ..

A setback occurred in Madras when in 1910 some cattle of country, Australian and cross breeds were inoculated with very bad results. The report of a Committee of Enquiry unfortunately became widely disseminated and the method was voted dangerous with the result that officers have not wanted to court disaster and have hesitated to break away from the safety of serum alone.

The high mortality was due to two distinct causes, *viz.*, the use of an insufficient dosage of serum and the inoculation of animals already infected with the disease. As the inoculations were pioneer work and the cattle were largely of imported strains, the infected herd should have been inoculated with serum alone as then the mortality would not have caused surprise. Actually it would have been the same, as it is unlikely that the virus given for the simultaneous inoculation affected the results in the animals already infected.

The insufficient dosage of the serum used was unfortunate as had three or four hill bull units been employed instead of two units, the result would have been a brilliant success in the uninfected animals and the method would have been successfully launched in India.

At that time no experience had been gained in the dosage required for imported cattle, and it was considered that double the dose was an enormous increase in view of the fact that the natural mortality in Kumaun Hill bulls was about the same as for the Australian cattle. Further it was then generally believed that it was necessary to balance the dose of serum so that a good

reaction would result and that the immunity conferred would be in proportion to the reaction obtained.

In this connection it is of interest to note that mortality to the disease cannot be taken as an indication of the minimum dose of serum required for protection.

Kumaun Hill cattle show a natural mortality of ninety-eight per cent. or over and the serum issued by this Laboratory is always proved before issue to be protective to them in a dose of 90 c.c. per 600 lb. body weight.

Bulls of the Ayreshire breed imported by the Military Dairy Department, which probably do not show a higher natural mortality, have been found to require a dose of 300 c.c. per 600 lb. body weight.

The Muktesar Laboratory has been working on the question and for some years has supervised the immunization of the cattle of the Military Dairies of which a large percentage are of highly susceptible imported and cross-bred strains. At first an officer of the Royal Army Veterinary Corps was deputed to carry out the inoculation and later when during the War no such officers were available, it took over the work and employed a special staff of two Deputy Superintendents who toured around in the cold weather from dairy to dairy immunizing the herds.

Finally last year the Royal Army Veterinary Corps took over the work again. The result is that we now have a great deal of practical experience both of the possibilities and of the limitations of the method.

From 1917 up to the date of handing over the inoculation work to the Royal Army Veterinary Corps on the 1st of January, 1921, five thousand and twenty-six animals were inoculated with a mortality of 0·8 per cent.

The history of the method in India has been a start with small doses of serum with bad results and a gradual increase in the dosage, particularly for highly susceptible animals, until there is now practically no danger of losing animals from rinderpest contracted from the inoculation.

The results of the experiments detailed on page 132 show that even when relatively large doses of serum are used, the virulent blood infects and confers active immunity while the serum masks the reaction. Hence it appears that there is no necessity to gauge the dosage accurately according to the susceptibility of the animals for fear of the serum overpowering the virus. The danger is when the dose of serum is insufficient to protect the animal.

(*b*) In other countries.

The serum simultaneous method of inoculation has been carried out on a large scale in the field both in Egypt and other parts of Africa. Rinderpest

reappeared in Egypt in 1903 after an absence of more than 20 years and caused very heavy losses. From 1904 to 1912 the *serum alone* method was employed for combating the disease but the results obtained were not satisfactory.

In this connection Littlewood states[18]: "From a long experience I came to the conclusion that serum could not be considered an efficient means for stamping out cattle plague (Rinderpest); it acted frequently as a temporary check and prevented for the time being the disease extending."

A Commission was appointed in Egypt in 1912 to study the best means of fighting the disease. Todd and White[14], both of whom were members of the Commission, state in connection with work on the duration of serum immunity: "In view of the unfavourable opinion of all the members of the Commission on the 'Serum Alone' method for dealing with the disease in Egypt, these experiments were discontinued."

The serum simultaneous method was put into practice in 1912 and up to the end of March 1920, as shown in the following table, 472,776 cattle were inoculated with a mortality of 0·98 per cent. from all causes.

Littlewood says in connection with the efficiency of the method[19]:— "The Government has now at its disposal the means of controlling cattld plague, *i.e.*, double inoculation of cattle systematically."

TABLE VI.

Showing total number of cattle inoculated by the serum simultaneous method in Egypt from 1912 *to* 1920, *with the percentage of losses from rinderpest and other diseases.*

Year	Number of cattle inoculated	Deaths			
		Rinderpest	Other diseases	Total	Percentage
1912	7,315	61	12	73	1·00
1913	178,495	569	1,452	2,021	1·13
1914	104,916	166	1,955	2,121	2·02
1915	4,833	29	....	29	0·60
1916	1,134	3	....	3	0·26
1917	80,036	6	2	8	0·01
1918	43,228	100	5	105	0·24
1919	6,709	110	....	110	0·16
1920	46,110	99	72	171	0·37
TOTAL	472,776	1,143	3,498	4,641	0·98

The degrees of susceptibility of Egyptian cattle to the disease are very similar to those of Indian cattle and vary from highly susceptible animals to those possessing a large amount of immunity.

During the recent outbreak of rinderpest in Central Africa, close on one hundred thousand cattle were inoculated by this method. The mortality from all causes following the inoculation was about five per cent. and it was caused mainly by East Coast Fever (*Theileria Parva* infection) and Texas Fever (*Piroplasma bigeminum* infection).

Gray, Principal Veterinary Officer, Union of South Africa, who was chief of the Rinderpest Commission in Central Africa (1918-1919), remarks[20]. "Generally speaking the results have been satisfactory and the mortality following the inoculation, except in cases in which complicating factors crept in which could hardly have been anticipated or combated, was relatively small."

"It must be admitted on all sides that the present abundance and the generally healthy condition of the large numbers of cattle found to-day in some of the areas in which the Commission worked, is the most satisfactory proof that the results obtained have been so good or better than might have been expected, especially if the conditions in these areas are compared with those now obtaining in others through which the disease swept without interference and where the majority of the cattle have died from its ravages."

It must be remembered in considering this (Central African) campaign against the disease that the conditions in which the work was carried out were entirely different to those obtaining in India.

It was instituted for the purpose of making an immune belt about 200 miles long and 5 miles wide at its narrowest, to the North of Rhodesia and Nyassaland, and to stamp out the disease locally so that there should be no danger of its spreading into South Africa.

Most of the high mortality which resulted was caused by moving large numbers of cattle for the purpose of the inoculation into concentration camps. It was found later that East Coast Fever was enzootic in the areas in which some of these were situated and through which some of the cattle passed.

III. The duration of the immunity conferred by serum simultaneous inoculation.

Active immunity is conferred by this method of inoculation and all investigators consider that it is of long duration.

Holmes[1] carried out experiments to ascertain the duration of the immunity. He tested animals so inoculated after an interval of three months and found that they were immune.

Kolle and Turner[21] found that animals they tested were immune five months after being immunized, and Nicolle and Adel Bey[5] found that animals were immune for long periods.

In the inoculations carried out in the Indian Military Dairies under the direction of the Muktesar Laboratory, animals that failed to react to the immunization were tested the following year by the inoculation of virulent blood alone and always proved to be immune.

Mason and Piot Bey in Egypt have carried out a series of valuable experiments on this question. In June 1912, one thousand six hundred and seventy cattle of the Domains Administration were inoculated by the serum simultaneous method. A certain number of these cattle have been tested with virulent blood alone each subsequent year. The last lot tested proved to be immune six years and four months after the original inoculation in 1912.

It is reasonable to assume therefore that this method confers an immunity for at least the average life of a beast.

IV. A description of some experiments designed to ascertain if it is possible to confer active immunity with certainty by the serum simultaneous method of inoculation when relatively large doses of serum are used.

Owing to the increasingly large numbers of well-bred dairy stock which are being imported yearly into India, it is most important that the risk of death from the serum simultaneous method of inoculation should be eliminated as far as possible. Imported stock are highly susceptible to rinderpest and the risks following the inoculation are consequently greater than in animals possessing a partial immunity.

Originally it was considered that the dose of serum must be regulated according to the susceptibility of the animal so that a good rinderpest reaction would result from the simultaneous inoculation of virulent blood and that the degree of active immunity would be in proportion to the severity of the reaction.

Holmes[1] proved that this is not the case as with single, double and treble doses of serum; he was able to confer immunity that was serviceable three months later.

We have considered it profitable to go a little further and ascertain if with even larger doses of serum, the same results would be obtained. If this proved successful the scope of the method would be very considerably widened as calves under nine months old, cows more than three months in calf and cows in full milk yield, which have not

been inoculated in this way up to the present in India, could then be dealt with.

The inadvisability of so treating these animals has been found to be a great drawback, as it has always meant that when a herd was inoculated, they had to be left over for the future and in actual practice some cows have escaped for a year or two as they were always either in calf or in milk when the inoculations were performed.

Details of the experiments.

A number of Kumaun Hill bulls (Table VII) were immunized with brew B of serum (Table III) by giving them four, six, eight, and ten times a single dose of the serum and inoculating them the next day with 1 c.c. of virulent blood. The reactions were not noted as they were isolated in a healthy outkraal away from all risk of subsequent infection and only their own attendants were allowed near them.

Ninety days after the immunization, 10 c.c. of virulent blood was inoculated into each and accurate charts were then maintained for three weeks.

Table VII.

A

Result of serum simultaneous inoculation giving four *times normal dose of serum.*

Retested with 10 c.c. of virulent blood from Hill bull No. 1770, temperature 105·0°F., vesicles 5th day of attack.

Number of animal hill bull	Weight lb.	Actual dose of serum c.c.	Virus c.c.	Retested after an interval of days	Virus c.c.	Result
1777	395	158	0·5	90	10	No reaction.
1778	335	134	0·5	90	10	,,
1779	465	186	0·5	90	10	,,
1780	355	142	0·5	90	10	,,
1781	342	137	0·5	90	10	,,
1782	338	135	0·5	90	10	,,
1802	360	144	0·5	90	10	,,

TABLE VII.—(*contd.*)

Controls.

Hill bull No. 1773, temperature 104·6°F., vesicles 5th day of attack.
Hill bull No. 1775, temperature 104·8°F., vesicles 4th day of attack.

B

Result of serum simultaneous inoculation giving six *times normal dose of serum.*
The same virulent blood and controls as in A.

Number of animal hill bull	Weight lb.	Actual dose of serum c.c.	Virus c.c.	Retested after an interval of days	Virus c.c.	Result
1783	375	225	0·5	90	10	No reaction.
1784	400	240	0·5	90	10	,,
1785	385	231	0·5	90	10	,,
1787	280	168	0·5	90	10	,,
1793	300	180	0·5	90	10	,,
1803	340	204	0·5	90	10	,,
1804	355	213	0·5	90	10	,,

C

Result of serum simultaneous inoculation giving eight *times normal dose of serum.*
The same virulent blood and controls as in A.

Number of animal hill bull	Weight lb.	Actual dose of serum c.c.	Virus c.c.	Retested after an interval of days	Virus c.c.	Result
1786	285	228	0·5	90	10	No reaction.
1788	335	268	0·5	90	10	,,
1789	334	268	0·5	90	10	,,
1790	390	312	0·5	90	10	,,
1791	440	352	0·5	90	10	,,
1792	506	405	0·5	90	10	,,
1801	362	290	0·5	90	10	,,

TABLE VII.—(*concld.*)

D

Result of serum simultaneous inoculation giving ten *times normal dose of serum.*
The same virulent blood and controls as in A.

Number of animal hill bull	Weight lb.	Actual dose of serum c.c.	Virus c.c.	Retested after an interval of days	Virus c.c.	Result
1794	172	172	0·5	90	10	No reaction.
1795	350	350	0·5	90	10	,,
1796	381	381	0·5	90	10	,,
1797	345	345	0·5	90	10	,,
1798	360	360	0·5	90	10	,,
1799	346	346	0·5	90	10	,,
1800	358	358	0·5	90	10	,,

CONCLUSIONS.

1. Four, six, eight and ten times a single dose of anti-rinderpest serum failed to prevent infection with rinderpest when virulent blood was inoculated the following day.

2. Susceptible animals can safely be immunized against rinderpest even when relatively large doses of serum are used, as proved by failure to react to an inoculation with virulent blood alone ninety days later.

3. There is no necessity to gauge the dose of serum according to the susceptibility of the animal for fear of the serum preventing infection.

V. The possibility of introducing serum simultaneous inoculation into india for general use in the districts.

Leaving aside the question of religious prejudice amongst a section of the population, the conditions in India are ideal for the application of this method of inoculation against rinderpest.

In this connection Ware [5] states: "It seems possible that at least some of them (the owners) would not object to simultaneous inoculation, provided the virus was obtained from outside and they did not see the animals bled. It would be a great advantage therefore and save much time and serum by avoiding further reinoculation, if arrangements could be made to give the animals of those people willing for it, a dose of virulent blood simultaneously with the serum."

Unequalled facilities exist for training the staff in the technique of the method. Anti-rinderpest serum of high potency is already being prepared on a very large scale in the country.

The production of virus which is one of the most important factors in the successful execution of the inoculation would require careful consideration. For the initial inoculations, the virus could be obtained on a large scale during the winter months from the branch laboratory at Bareilly. Later on as the scope of the inoculations increase, virus-producing centres could be started in each province or if the scheme for the establishment of a second laboratory in Southern India materializes, this would be able to prepare all the virus that is required for most of the southern area.

The inoculation of virus producers in the districts at the scene of operations is not practicable for many reasons and further it might offend the religious principles of certain classes.

As a beginning for ordinary district purposes, the method could be employed only at the site of outbreaks of the disease so that fresh centres of infection would not be manufactured. This would be as much as the staff available could deal with at least for a year or two.

As it is essential in India that any method for the control of disease should be as popular as possible and more especially when bovines are concerned, the start should be on a small scale in each province and compensation should be given to the owners of animals that die as the result of the inoculation.

Sufficient experience of the method has now been gained in India and other countries to warrant this step.

If ten thousand rupees were allowed to each province for the first year or two for the grant of compensation until the method became popularized, it would go a long way.

Taking all classes of cattle and buffaloes, young stock, cows, etc., such as met with in villages, an average price of seventy-five rupees per head is probably a liberal estimate.

In the serum alone inoculations reported by the different provinces during the last five years the mortality after inoculation has been 0·43 per cent. As the inoculations are carried out at the site of outbreaks of the disease this covers the deaths in animals inoculated with serum after it is too late to save life but before it is possible to say that the animal is infected.

In view of this contingency the figure quoted would appear to be very low but it is probably due to the general low susceptibility of the animals dealt with.

When carrying out simultaneous inoculation, precautions should of course always be taken that animals already visibly affected with the disease are left uninoculated. The inoculation of virus into these animals would not affect the course of the disease, but owners would be ready to blame it.

Taking everything into consideration the mortality should not exceed one per cent., so ten thousand rupees should be sufficient to compensate for about fifteen thousand inoculations.

The speed with which the inoculation stops the infection would more than compensate for the mortality, as the longer the infection remains, the more chance there is of other herds contracting the disease.

It is of course unnecessary to point out the economic loss which results from an outbreak of rinderpest. This makes itself felt in the loss of young stock from abortions and from the death of in-calf cows, from loss of milk supply, dislocation of agricultural operations, especially at critical seasons, and indirectly in many other ways apart from the actual value of the animals which die.

REFERENCES.

1. HOLMES .. Report of the Research Work of the Imperial Bacteriological Laboratory, Muktesar, 1908-09. *Indian Civil Veterinary Department Memoirs*, No. I, p. 69.
2. DANYSZ and BORDET, quoted by HUTCHEON .. *Journ. of Comp. Path. and Therap.*, Vol. XV., Dec. 1902, p. 316.
3. SPREULL, quoted by HUTCHEON .. (See 2).
4. STOCKMAN. .. .. *Report of the Transvaal Department of Agriculture*, 1904.
5. NICOLLE and ADEL BEY.. *Annales de L'Institut Pasteur*, 1899.
6. RUEDIGER .. .. Observations on Cattle Plague in the Philippine Islands and the Methods Employed in Combating it. *The Philippine Journ. of Science*, Vol. IV, No. V., Sect. B., Med. Sciences Oct. 1909, p. 381.
7. JOBLING .. .. Preliminary Report of the Study of Rinderpest of Cattle and Carabaos in the Philippine Islands. *Bureau of Govt. Lab. Dept. of Interior, Manilla*, 1903.
8. HEAD .. .. Cattle Plague in the Sudan. *Journ. of Comp. Path. and Therap.*, 1906, p. 12.
9. WARD and WOOD . Experiments on the Efficiency of Anti-Rinderpest Serum. *Bull.* No. 19, *Govt. of Phil. Is., Dep. of Pub. Instr., Bureau of Agri.*, 1912.
10. WARD and WOOD .. Simultaneous Method of Inoculating Cattle and Carabaos with Serum from animals that have been recently immunized. *Phil. Journ. of Science*, Vol. IX, No. I, Sect. B, Trop. Med., Feb. 1914.
11 SHILSTON .. .. Rinderpest. Preparation of Anti Serum. *Bull.* No. 64, *Agric. Res. Inst., Pusa, India*, 1916.

12. SHILSTON .. .. Protective Inoculation of Stock in India. *The Agri. Journ. of India*, Vol. II, Part 2, April 1916.

13. POOL and DOYLE .. The Preparation of Anti-Rinderpest Serum using Animals of Moderate Susceptibility as Virus Producers. *Bull.* No. 129. *Agri. Res. Inst., Pusa, India*, 1921.

14. TODD and WHITE. .. Experiments on Cattle Plague. Reported from The Hygienic Inst., Dep. of Pub. Health, Cairo, 1914-15.

15. WARE .. .. Proceedings of the First Meeting of Veterinary Officers in India, Appendix C, 1919.

16. POOL .. .. The Serum Simultaneous Method of Inoculation against Rinderpest. *Bull.* No. 120, *Agri. Res. Inst., Pusa, India*, 1921.

17. .. .. .. *Proceedings of the First Meeting of Veterinary Officers in India*, 1919, p. 29.

18. LITTLEWOOD .. *Annual Report, Ministry of the Interior, Dep. of Pub. Health. Vety. Serv., Egypt*, 1912.

19. LITTLEWOOD .. *Annual Report, Min. of Agric., Egypt, Vety. Serv.*, 1914.

20. GRAY .. .. Report of the Rinderpest Expedition. Union of South Africa, 1920.

21. KOLLE and TURNER .. *Zeitschrift fur Hygiene*, Vol. XXIX, 1898.

22. MASON and PIOT BEY .. *Annual Report, Min. of Agri., Egypt, Vety. Serv.*, 1919-20

ETIOLOGY OF EQUINE CONTAGIOUS ABORTION IN INDIA.

BY

T. M. DOYLE, F.R.C.V.S.,

Veterinary Officer, Imperial Bacteriological Laboratory, Muktesar.

[Received for publication on 8th November, 1921.]

THIS disease has attracted a great deal of attention both in Europe and America during the last decade. From the point of view of economic importance, equine abortion cannot be considered to be in the same rank as bovine abortion. The question of milk supply while of primary importance in the cow does not arise in the case of the mare. Retained placenta and sterility which are a common sequel of bovine abortion are rarely met with in equine abortion.

Some writers on contagious abortion describe a very severe form of the disease in which retained placenta, metritis and death are a common sequel of the abortion. We have not met with any of these complications either in naturally or artificially infected animals. In our experience the abortion occurs without the slightest difficulty and without in any way impairing the health of the mare. Nevertheless the disease causes heavy financial loss, and owing to the fact that immunity is probably not so readily acquired by the mare as by the cow, the equine disease is more difficult to eradicate from a farm.

The introduction of the disease into a stud causes heavy losses from abortion and further because many full-time foals of infected mares born alive and apparently healthy develop joint-ill and die within a few days of birth.

In 1913 Good and Corbett[1] in America isolated a bacillus from aborted fœtuses to which they gave the name *Bacillus abortivus equinus*. In 1916

Good and Smith[2] suggested the name should be changed to *B. abortivo equinus* as " *B. abortivus equinus* is a trinomial designation contrary to the accepted rule for bacterial nomenclature." They (Good and Corbett) showed that an intravenous inoculation of 2 c.c. of emulsion of this organism caused abortion in a mare, the period of incubation being 10 days.

These investigators were the first to describe the very characteristic growth of the organism on the surface of agar, the medium after twenty-four hours' incubation having a dry wrinkled appearance, the intersecting lines being raised above the surrounding surface.

Meyer and Boerner[3] also in 1913 identified the same organism as described by Good and Corbett. These authors showed that the *Bacillus abortivo equinus* fulfilled all the requirements of organisms belonging to the paratyphoid B group and that its growth on artificial media could be used for differentiation. With cultures of the organism they were able to produce abortion in a cow, a goat, and a sow.

Dassonville and Rivière[4] in 1913 described a bacillus which they isolated from three aborted fœtuses. From the description of the growth of the organisms on agar, it was most probably the *B. abortivo equinus.*

In 1914, Neffs[5] isolated the bacillus of specific abortion of donkey mares in the Belgian Congo. From the results of agglutination tests carried out by Bruynoghe this organism would appear to be identical with the bacillus of equine abortion in Europe. Van Saceghem [a] in 1920 refound this bacillus in the organs of aborted donkey fœtuses in the Belgian Congo. An attempt by Van Saceghem to infect a pregnant horse mare per vaginum with the fluids from the membranes of an aborted donkey fœtus failed. He states this organism is specific for donkey mares only. As " Annales de Médicine Vétérinaire " in which the original article appeared is not available it is impossible to criticise his conclusions.

McFadyean and Edwards[6] in 1916 isolated the *B. abortivo equinus* from the organs of aborted foals, and also from the joint-fluid of foals which died of joint-ill. These authors gave a detailed description of the wrinkling presented by surface growths of the organism on agar, pointing out, however, that this characteristic appearance is not presented by every surface growth of the bacillus on agar and if growth is slow the wrinkled appearance may be delayed or permanently absent.

The *B. abortivo equinus* has been isolated from aborted fœtuses in other parts of the world, besides those mentioned, and the consensus of opinion is that the organism is the commonest cause of equine contagious abortion.

The *Bacillus abortivo equinus* as a possible cause of joint-ill in foals.

The organism has been stated to be also the cause of joint-ill in foals. This view was based on the fact that the bacillus has been obtained in pure culture from the joint-fluid and internal organs of foals affected with joint-ill. In most of these cases, however, contagious abortion was present in the establishments from which the foals came.

When contagious abortion exists in a stud a percentage of full-time and apparently normal foals develop symptoms of joint-ill shortly after birth, and from these cases the *B. abortivo equinus* can often be obtained in pure culture, and must therefore be regarded as a possible cause of joint-ill.

Intravenous injection of emulsion of the organism has on a couple of occasions at this Laboratory given rise to severe arthritis in pony mares (Nos. 12 & 4) used for experimental purposes. McFadyean and Edwards[6] report similar occurrences in horses employed for the preparation of anti-abortion serum.

In 1914, Schofield[7] investigated an outbreak of joint-ill in foals in Canada, and isolated a bacillus which he pointed out bore a close resemblance to the *B. abortivo equinus.* From the rapidity with which the symptoms developed after birth he was of the opinion that infection took place during the intra-uterine stage. Most investigators are, however, of the opinion that joint-ill which arises independently of specific abortion is a post-natal infection, but it is possible that those cases which are caused by the *B. abortivo equinus* may have a pre-natal origin. In many studs cases of joint-ill appear yearly although contagious abortion has never occurred.

The foals of certain mares are said to be more liable to the disease than those of other mares kept under identical conditions on the same premises.

According to Edwards[3] very few cases of joint-ill in foals in England are associated with specific abortion in mares.

From the recent researches of Magnusson[9] in Sweden and McFadyean and Edwards[10] in England it appears that fifty per cent. of cases of joint-ill are caused by a streptococcus infection, twenty-one per cent. by the *B. nephriditis equi* (*B. viscosum equi-Magnusson*) and twenty-one per cent. by organisms of the *B. coli communis* type. From these results it must be concluded that the *B. abortivo equinus* is the causal agent of joint-ill in only a very small percentage of cases and probably only when contagious abortion is co-existent in the stud.

The disease in India.

The disease is probably widely spread in parts of India, at least in Northern India, but it is difficult to get any reliable details on this point. In India, with the exception of a couple of clinical reports of outbreaks in the Punjab, no work has been carried out on the disease and its etiology is still obscure.

The disease is usually introduced into breeding establishments by the purchase of brood mares at fairs, many of which are either barren or have recently aborted, as Indian owners do not usually dispose of good mares. Very often the reason for a zemindar selling a mare is that she has aborted and he wants to get rid of her for that reason.

In 1909, Webb[11] reported an outbreak of abortion amongst donkey mares at the Mona Remount Depôt, Punjab. A stud of pony mares closely adjacent to the donkey mares remained free of the disease, although there was free communication between the attendants of both herds.

In 1921, Branford and Doyle[12] published an account of a severe outbreak of the disease amongst pony and donkey mares at the Government Cattle Farm, Hissar, Punjab.

In this outbreak twenty-four donkey mares and four pony mares aborted.

The present work was undertaken with the object of investigating the following questions :—

(1) The etiology of the disease.

(2) If the causal organism of the disease in India is the same as in Europe and America.

(3) If the same organism is responsible for the disease in pony and donkey mares.

The necessary material for the investigation was obtained through the courtesy of Mr. R. Branford, Superintendent, Government Cattle Farm, Hissar, Punjab.

Five aborted donkey fœtuses and two aborted pony fœtuses were obtained immediately after expulsion.

Broth and agar tubes were inoculated from the heart blood, liver, kidney, spleen and stomach and in every case the same organism was obtained in pure culture. Smears from the fœtal organs showed the organism in abundance.

The organism isolated agrees in morphology, cultural characteristics and staining properties with those described by American and English observers

for the *B. abortivo equinus*. The organism isolated from pony fœtuses agrees in all particulars with that obtained from donkey fœtuses.

Cultures of the organism are capable of producing abortion in both pony and donkey mares by intravenous, alimentary and intravaginal infection, the organism being recovered in most cases from the fœtal envelopes and organs of the aborted fœtus.

The disease has been produced in a pony mare (No. 4) by the intravenous injection of emulsion of the organism isolated from an aborted donkey fœtus, and in a donkey mare (No. 21) by the inoculation of the organism from an aborted pony fœtus.

Serum from a pony mare which has aborted gives a positive agglutination reaction with the bacillus obtained from a donkey fœtus and *vice versâ*.

We are therefore of the opinion that the same organism is the cause of the disease in both pony and donkey mares.

Etiology.

The causal organism is a short thick pleomorphic bacillus, often resembling a coccus, from 0·5 to 1μ long, 0·2 to 0·5μ in width. Large forms up to 3μ are frequently observed. Stains well by any of the simple aniline dyes.

In films the organism shows a tendency to stain at the ends leaving a small unstained central portion. It does not stain by the methods of Gram, Gram-Weight, or Claudius.

Cultural characteristics.

Agar. The organism grows well showing the characteristic wrinkling described by the American and English observers. Sometimes only a few small wrinkles are present at the drop of water of condensation, at other time the whole surface shows pronounced wrinkling. In some tubes there is no wrinkling, the growth appearing as a greyish white slimy film which can easily be removed from the surface of the medium. There is less tendency to wrinkling with the bacillus of donkey origin. Many tubes, however, show the characteristic growth, which is identical in appearance with that presented by the pony organism.

Broth. The organism grows well turning the medium cloudy in twenty-four hours. A pellicle occasionally forms after a few days' incubation which on slight agitation of the tube falls in flakes to the bottom.

Potato. On ordinary potato there is no growth. On potato treated with an alkaline solution growth usually occurs, but is scanty. There is nothing characteristic about the growth.

Gelatine. This medium is not liquefied.

Milk. Medium is not coagulated, the end of the reaction is alkaline.

Petroff's egg medium. The organism grows well and wrinkling of the surface of the medium is sometimes observed.

The organism grows with or without oxygen but in the latter case the growth is scanty.

In order to confirm the cross-agglutination tests the mare and donkey strains of the organism were tested on certain carbohydrates to see if any difference could be observed.

A lemco (1 per cent.), peptone (1 per cent.), soda bicarbonate (0·5 per cent.), litmus (10 per cent.) solution was used, together with 1 per cent. of the carbohydrates.

	Pony	Donkey
Mannite	A + G	A + G
Glycerine	Slightly acid	Slightly acid
Lactose *	A + G	A + G
Glucose	A + G	A + G
Galactose	A + G	A + G
Levulose	A + G	A + G
Inulin	....	....
Dextrose	A + G	A + G

* Very small amount of acid and gas
A + G = Acid and gas.

Read at twenty-fourth and forty-eighth hours. Incubated at 37°C.

These were the only sugars available.

These results are slightly different from those obtained by Meyer and Boerner[3], who found that the organism does not ferment lactose.

Good and Corbett[1] found, however, that the organism does ferment lactose with the production of a trace of acid and gas.

There is no production of indol.

A second series of tests were carried out similar to previous but using decolourized acid fuchsin, as recommended by Holman,[13] instead of litmus as an indicator. This method gave very satisfactory results.

Tenacity. Suspension of *B. abortivo equinus* washed off in saline solution from a forty-eighth hour agar growth was killed in eight minutes at 60°C. The

organism was not killed in 45 minutes at 55°C. Continuous subculturing causes a marked decrease in the virulency of the organism.

Bacillus abortivo equinus on plain agar. (Actual size.)

The agglutination test. This test has proved to be most reliable for the diagnosis of cases of natural infection with the *B. abortivo equinus.*

In artificially infected animals it has not, however, given the same uniform results. In animals infected by the intravenous route the test has invariably given a positive result and in high dilution, but in animals which have aborted as the result of infection by the alimentary or intravaginal tracts the results have not been reliable.

In those cases in which the test gave a negative result, the inoculation of culture tubes showed the infection to be localized.

The lowest dilution of serum causing complete agglutination that can be regarded as a positive result is 1/400.

Meyer and Boerner record a healthy horse with an agglutination titre of 1/360.

De Jong[14] estimated the highest agglutination titre of normal serum to be 1/300, and this agrees with the results obtained by Good and Corbett[1].

The highest dilution at which we obtained complete agglutination with normal serum was 1/200.

In the following tests the serum was obtained from pony and donkey mares which had become naturally infected during the outbreak at Hissar. The organism used was of pony origin for ponies and donkey origin for donkeys.

The donkey stallions shown in the list are two of the stud stallions from the Government Cattle Farm, Hissar. In the outbreak at Hissar there was no evidence pointing to the stallions as disseminators of the disease.

In the tests emulsion of the bacillus suspended in carbolized saline solution was made from a twenty-four-hour-old agar growth. The emulsion was standardized by the opacity method recommended by Seddon[15]. Results were read after twenty-four hours' incubation.

In each test control tubes with saline solution were used.

Agglutination tests with the bacillus isolated from cases of contagious abortion at Hissar.

Source of serm	1/100	1/200	1/400	1/600	1/800	1/1000	1/2000
Aborting pony mare	++	++	++	++	++	++	+
,, ,,	++	++	++	++	++	+	S
Aborting donkey mare	++	++	++	++	++	+	—
,, ,, ,,	++	++	++	++	++	++	+
,, ,, ,,	++	++	++	++	++	+	..
,, ,, ,,	++	++	++	++	++	+	S
Healthy donkey stallion	—	—	—	—	—	—	—
,, ,, ,,	—	—	—	—	—	—	—
,, pony mare	++	++	—	—	—	—	—
,, ,, ,,	+	+	—	—	—	—	—
,, donkey mare	++	+	—	—	—	—	—

+ + = Agglutination and clearing
+ = Agglutination.
S = Slight agglutination.
— = No agglutination.

INOCULATION EXPERIMENTS.

Four rabbits and four guinea-pigs were inoculated (two of each subcutaneously and two of each intraperitoneally) with emulsion of the organism of pony and donkey origin.

Organism of pony origin is shown by a (P)

Organism of donkey origin is shown by a (D)

Rabbit No. 1.—0·5 c.c. of emulsion (P) subcutaneously. Killed 60th day. Healthy.

Rabbit No. 2.—0·5 c.c. of emulsion (D) subcutaneously. Killed 60th day. Healthy.

Rabbit No. 3.—0·5 c.c. of emulsion (P) intraperitoneally. Killed 70th day. Healthy.

Rabbit No. 4.—0·5 c.c. of emulsion (D) intraperitoneally. Killed 70th day. Healthy.

Guinea-pig No. 2.—0·5 c.c. of emulsion (D) subcutaneously. Killed 70th day. Healthy.

Guinea-pig No. 4.—0·5 c.c. of emulsion (P) subcutaneously. Killed 70th day. Healthy.

Guinea-pig No. 5.—0·5 c.c. of emulsion (P) intraperitoneally. Killed 70th day. Healthy.

Guinea-pig No. 6.—0·5 c.c. of emulsion (D) intraperitoneally. Killed 70th day. Healthy.

Conclusion. From these results it may be concluded that no diagnostic reaction results from the inoculation of guinea-pigs or rabbits with the *B. abortivo equinus* similar to the reaction which usually occurs in guinea-pigs from the inoculation of the bacillus of bovine abortion.

Intravenous inoculation of rabbits.

Rabbit No. 17.—Inoculated intravenously with 0·01 c.c. of forty-eight-hour-old broth culture of the organism (D). Died in two days and eighteen hours.

Rabbit No. 18.—Inoculated intravenously with 0·01 c.c. of forty-eight-hour-old broth culture of the organism (P). Moderate reaction. Recovered.

Rabbit No. 106.—Inoculated intravenously with 0·1 c.c. of forty-eight-hour-old broth culture of the organism (D). Moderate reaction. Recovered.

Rabbit No. 91.—Inoculated intravenously with 0·1 c.c. of forty-eight-hour-old broth culture of the organism (P). Died 6th day.

Rabbit No. 93.—Inoculated intravenously with 0·1 c.c. of forty-eight-hour-old broth culture of the organism (P). Died in 20 hours.

Rabbit No. 94.—Inoculated intravenously with 0·1 c.c. of forty-eight-hour-old broth culture of *B. abortivo equinus* (D). Died in 24 hours.

In every case of death the organism was isolated from the blood and the organs.

Conclusion. The *B. abortivo equinus* is pathogenic for rabbits in doses of 0·1 c.c. of cltuure inoculated intravenously.

Experiments on pregnant rabbits and guinea-pigs.

Rabbit No. 10.—Pregnant. Given with food 25 c.c. of amniotic fluid from an aborted pony fœtus. Aborted 4th day.

Rabbit No. 40.—Pregnant. Inoculated subcutaneously with 1 c.c. of twenty-four-hour-old broth culture of organism (P). Aborted 4th day.

Guinea-pig No. 15.—Pregnant. Inoculated subcutaneously with 1 c.c. of twenty-four-hour-old broth culture of organism (P). Aborted 10th day.

Guinea-pig No. 18.—Pregnant. Inoculated subcutaneously with 1 c.c. of forty-eight-hour-old broth culture of organism (D). Aborted 5th day.

Conclusion. Rabbits and guinea-pigs become infected by subcutaneous inoculation of culture of the *B. abortivo equinus.* The only feeding experiment on one pregnant rabbit gave a positive result.

Intravenous inoculation of pregnant pony and donkey mares.

Pony mare No. 12.—Inoculated intravenously on January 4th, 1921, with 2 c.c. of suspension of *B. abortivo equinus* (P) in saline solution from a twenty-four-hour-old agar growth. During the thirty-six hours following the inoculation the temperature rose to 104·4°F. and then gradually dropped to normal.

Aborted a six-month-old fœtus on January 14th. The period of incubation was 10 days. No premonitory symptoms were observed although the mare was kept under close observation. The after symptoms were dullness, capricious appetite and slight blood-stained discharge from the vulva which disappeared without treatment in a couple of days.

The *B. abortivo equinus* was found in a pure state in the amniotic fluid, heart, liver and stomach of the fœtus.

This mare developed severe arthritis of the right shoulder-joint fourteen days after the injection of the emulsion. An abscess formed and burst. Tubes inoculated from the pus were contaminated.

The fœtal blood gave a negative reaction to the agglutination test.

Agglutination test of serum from pony mare No. 12.

	1/400	1/600	1/800	1/1000	1/2000	1/3000
Before injection	−	−	−	−	−	−
Four days after aborting	++	++	++	++	++	+
Serum from fœtus	−	−	−	−	−	−

Serum from pony mare No. 12 V. donkey bacillus.

	1/400	1/600	1/800	1/1000	1/2000	1/3000
Four days after aborting	++	++	++	++	+	−

Pony mare No. 4.—Inoculated intravenously on February 17th, 1921, with 2 c.c. of suspension of *B. abortivo equinus* (D) in saline solution from a twenty-four-hour-old growth on agar. As abortion did not take place this mare was given 5 c.c. of a twenty-four-hour-old-broth culture of *B. abortivo equinus* (D) on April 6th.

Aborted a six-month-old fœtus on April 16th.

This mare was presumably infected from the second inoculation therefore the period of incubation was ten days.

Cultures from the amniotic fluid, heart-blood, liver, stomach, spleen and kidneys of the fœtus all showed the *B. abortivo equinus* in pure culture.

A small quantity of thin yellow fluid was drawn from the udder and tested by the agglutination method with negative result.

Agglutination test.

	1/400	1/600	1/800	1/1000	1/2000	1/3000
Serum (of mare)	++	++	++	++	++	+
Fluid from udder	−	−	−	−	−	−

This mare developed a severe arthritis of the off stifle-joint fourteen days after the second inoculation, and four days after aborting.

Donkey mare No. 23.—Inoculated intravenously on January 4th, 1921, with 1 c.c. of suspension of *B. abortivo equinus* (D) in saline solution from a twenty-four-hour-old growth on agar. Within thirty-six hours of inoculation the temperature rose 2·5°F., remained high for three days and then dropped to normal.

She aborted a nine-month-old fœtus on January 25th. The period of incubation was twenty days. There were no premonitory symptoms and the temperature remained normal immediately before and after the act of abortion.

There was a slight watery discharge from the vulva which disappeared without any treatment after a couple of days. The mare did not come into milk.

Smears and cultures from the fœtal envelopes and the fœtal organs showed the *B. abortivo equinus* in pure culture.

Agglutination test of serum from donkey mare No. 23.

	1/400	1/600	1/800	1/1000	1/2000
Before injection	−	−	−	−	−
23 days after injection	++	++	++	++	+
106 days after injection	−	−	−	−	−

Donkey mare No. 11.—Inoculated intravenously on Jannary 18th, 1921, with 2 c.c. of suspension of *B. abortivo equinus* (D) in saline solution from a twenty-four hours' growth on agar. The temperature rose to 104·4°F. in the succeeding twenty-four hours and then gradually dropped to normal. She aborted a six-month-old fœtus on February 5th. The period of incubation was 18 days.

Smears and cultures from the fœtal organs showed the *B. abortivo equinus* in abundance and in pure culture.

Agglutination test of serum from donkey mare No. 11.

	1/400	1/600	1/800	1/1000	1/2000
Before injection	−	−	−	−	−
27 days after injection	++	++	++	++	++
76 days after injection	++	++	++	++	++

Serum from donkey mare No. 11. V. pony bacillus.

	1/400	1/600	1/800	1/1000	1/2000
27 days after injection	++	++	++	++	++

Donkey mare No. 21.—Inoculated intravenously on January 20th, 1921, with 2 c.c. of suspension of *B. abortivo equinus* (P) in saline solution from a twenty-four hours' growth on agar. Temperature rose 2·7°F. in the succeeding twenty-four hours and then dropped to normal. She aborted a five-month-old fœtus on February 7th. The period of incubation was eighteen days.

There were no symptoms shown either before or after the act of abortion. *B. abortivo equinus* was recovered in pure culture from all the fœtal organs.

Agglutination test of serum from donkey mare No. 21.

	1/400	1/600	1/800	1/1000	1/2000
Before injection	−	−	−	−	−
25 days after injection	++	++	++	++	++
74 days after injection	++	++	++	++	−

Pony gelding No. 233.—Inoculated intravenously on January 24th, 1921, with 10 c.c. of a forty-eight-hour-old broth culture of *B. abortivo equinus* (D).

The following morning the pony was off its feed, lying down and passing thin watery fæces. It became very weak and emaciated and death took place on the eighth day.

There was no rise of temperature following the inoculation as is usually the case.

Pony gelding No. 253.—Inoculated intravenously on January 24th, 1921, with 10 c.c. of forty-eight-hour-old broth culture of *B. abortivo equinus* (P). Died twelve hours after injection.

Two pony mares and three donkey mares were successfully infected by the intravenous inoculation of culture of the *B. abortivo equinus* and aborted after an average incubation period of ten days and nineteen days, respectively.

One of the pony mares (No. 4) was infected with the *B. abortivo equinus* of donkey origin.

One of the donkey mares (No. 21) was infected with the *B. abortivo equinus* of pony origin.

The serum of an infected pony mare (No. 12) agglutinated the bacillus of donkey origin and the serum from an infected donkey mare (No. 11) agglutinated the bacillus of pony origin.

In the only case tested the fœtal blood gave a negative reaction to the agglutination test although the *B. abortivo equinus* was cultivated from the fœtal organs.

The agglutination titre of one donkey mare (No. 23) had fallen below 1/400 when tested one hundred and six days after inoculation.

The intravenous inoculation of 10 c.c. of broth culture of the organism killed two pony geldings in twelve hours (No. 253) and eight days (No. 233), respectively.

FEEDING EXPERIMENTS.

Pony mare No. 3.—On January 14th, 1921, was fed with 250 c.c. of amniotic fluid from the aborted fœtus of pony mare No. 12. The temperature

rose 2° F. during the succeeding forty-eight hours and then gradually dropped to normal.

On February 13th (thirty days after feeding) blood was drawn and tested by the agglutination method with negative results.

On February 23rd the mare was fed with 100 c.c. of a forty-eight-hour-old broth culture of *B. abortivo equinus* (P). The temperature rose 3°F. during the succeeding forty-eight hours and then gradually dropped to normal.

On April 4th, she was fed with 430 c.c. of a forty-eight-hour-old broth culture (P). The temperature rose 1°F. during the following forty-eight hours.

On April 22nd the agglutination titre was 1/800.

On April 30th she was fed with 1,000 c.c. of a forty-eight-hour-old broth culture (P). The temperature remained normal. From this fact it would appear that the mare had acquired some immunity.

On June 15th, 10 c.c. of a forty-eight-hour-old broth culture was given intravenously to test if any immunity had been acquired. Temperature rose 3·2°F., following the injection and then fell to normal.

The mare aborted an eight-month-old fœtus on June 23rd, eight days after the injection.

The *B. abortivo equinus* was recovered in pure culture from all the fœtal organs.

The mare was most probably infected by the intravenous injection and not from any of the material given with the food.

Agglutination test of serum from mare No. 3.

Two days after abortion.

1/2000	1/3000	1/5000	1/10000
++	++	++	+

Although 1,530 c.c. of broth culture together with 250 c.c. of amniotic fluid rich in the organism were given per os abortion was not brought about and later after a period of :—

152 days after the first feeding.
122 ,, ,, ,, second ,,
112 ,, ,, ,, third ,,
72 ,, ,, ,, fourth ,,
46 ,, ,, ,, fifth ,,

Abortion was caused by an intravenous inoculation of culture.

Pony mare No. 7.—On February 18th, 1921, was fed with 25 c.c. of a forty-eight-hour-old broth culture of *B. abortivo equinus* (P).

On February 26th, a premature living foal was born. It died twelve hours after birth. The period of incubation was eight-and-a-half days.

The *B. abortivo equinus* was obtained in pure culture from the heart, liver, spleen and kidneys of the foal. There were no macroscopic lesions in the foal. This mare came into milk and some was drawn for the agglutination test two days after the abortion took place. The milk clotted naturally and after twenty-four hours the whey had separated out.

Seddon[15] has shown that the bacterially produced whey from the milk of a cow which had aborted gave a positive agglutination reaction.

Reinhardt and Gauss[16] record a positive agglutination with cows' milk up to 1/1000.

Agglutination test of serum and whey from pony mare No. 7.

	1/400	1/600	1/800	1/1000	1/2000	1/3000
Whey	++	++	++	++	++	++
Serum	++	++	++	+	..	..

The serum of this mare tested sixty days after the feeding with the culture gave a negative reaction.

The whey from the milk of a healthy mare and a cow were used as controls, the result in each case was negative. Another sample from the same lot of whey (pony No.7) which had been standing at room temperature for sixty-eight days was retested and again gave a positive reaction up to 1/3000.

This mare was the only experimentally infected animal which came into milk after aborting, she dried up a few days after the death of the foal so unfortunately more milk could not be obtained. Obviously no reliance can be placed on the testing of one sample, but it is worth mentioning. The *B. abortivo equinus* could not be found in the milk either by microscopical examination or by the inoculation of culture tubes.

*Donkey mare D*1.—On February 17th, 1921, was fed with 25 c.c. of a forty-eight-hour-old broth culture of the organism (D).

She aborted a six-month-old fœtus on March the 23rd. The period of incubation was thirty-four days. Smears and cultures made from fœtal organs were negative. Blood drawn two days after the abortion occurred was negative to the agglutination test. The blood was again tested fifty-one days later with negative results.

The fœtal envelopes were not examined being torn and dirty, so there is the possibility that they were infected as in donkey mare D 2.

Donkey mare D 2.—On February 17th, 1921, was fed with 12 c.c. of a forty-eight-hour-old broth culture of *B. abortivo equinus* (P). Aborted a nine-month-old fœtus on February 23rd. The period of incubation was five-and-a-half days.

The *B. abortivo equinus* was cultivated from the fœtal envelopes, but all tubes inoculated from the fœtal organs were negative.

The blood was negative to the agglutination test.

The blood of both mares was tested by the agglutination test with negative results prior to *feeding with the culture.*

Conclusions. One pony mare and two donkey mares were successfully infected by feeding with culture of the *B. abortivo equinus.*

The agglutination test of the serum gave a negative result in both donkey mares. In the case of the pony mare, the agglutination titre of the serum was not very high, but milk from the same mare gave a very high titre.

One pony mare failed to become infected by the alimentary route, but aborted when infected intravenously.

Intravaginal infection.

Donkey mare No. 9.—10 c.c. of a forty-eight-hour-old broth culture of *B. abortivo equinus* (D) was injected into the vagina on the 12th August, 1921.

On the fifth day after injection she had a slight mucopurulent discharge from the vagina which lasted a couple of days and then disappeared. On the eighth day after injection the temperature rose to 103·6°F., remained high for forty-eight hours and then returned to normal.

She aborted a three-month-old fœtus on August 31st. The period of incubation was nineteen days.

The fœtal envelopes were not found.

The organism was recovered only from the liver of the fœtus. Tubes inoculated from the heart-blood, spleen and kidney, were sterile.

The agglutination test of the mare's serum gave a negative result two days after the abortion. The test was repeated at weekly intervals up to one month, the results were always negative.

Pony mare No. 15.—10 c.c. of a forty-eight-hour-old broth culture of *B. abortivo equinus* (P) was injected into the vagina on August 15th, 1921.

She remained quite normal after the injection, and fed well. There was no discharge from the vagina.

Aborted a three-month-old fœtus on August 27th. The period of incubation was twelve days.

The organism was isolated from the heart-blood, fœtal envelopes and fluid. Tubes inoculated from the liver were sterile.

This mare remained perfectly normal and showed neither rise of temperature nor vaginal discharge before or after the act of abortion.

The agglutination test gave a negative result two days after the abortion took place. When tested fourteen days after aborting the agglutination titre was 1/800.

Agglutination test of serum of pony mare No. 15.

	1/400	1/600	1/800	1/1000	1/2000
Two days after abortion	−	−	−	−	−
Fourteen days after abortion	++	++	++	+	−

Conclusion. Two mares (one pony and one donkey) were successfully infected by the intravaginal injection of culture of the *B. abortivo equinus* and aborted twelve and nineteen days respectively after infection.

THE INOCULATION OF FOALS WITH *Bacillus abortivo equinus.*

Mule foal No. 1. (age six months).—Inoculated intravenously on January 27th, 1921, with 5 c.c. of a forty-eight-hour-old broth culture of *B. abortivo equinus* (P). The same dose was repeated on January 17th and March 2nd. Except for a slight rise of temperature following each injection, this foal showed no symptoms.

Mule foal No. 2. (age seven months).—Inoculated intravenously on January 27th, 1921, with 5 c.c. of a forty-eight-hour-old broth culture of *B. abortivo equinus* (D). The same dose was repeated on February 17th and on March 2nd. The result was the same as in foal No. 1.

Pony foal No. 3. (age fourteen days).—Inoculated intravenously on April 13th, 1921, with 0·5 c.c. (in 5 c.c. of saline solution) of a forty-eight-hour-old broth culture of *B. abortivo equinus* (P). The only reaction was slight rise in temperature. On April 18th, 5 c.c. of a forty-eight-hour-old broth culture was inoculated intravenously. The foal died twelve hours later. No lesions were observed on post-mortem examination.

Pony foal No. 4. (age one month).—1 c.c. of a forty-eight-hour-old broth culture of *B. abortivo equinus* (P) in 20 c.c. of distilled water was given per os on March 30th, 1921.

The same dose was repeated on March 31st, and on April 1st. On April 15th, 18 c.c. of a forty-eight-hour-old broth culture was given per os. On April 18th, 40 c.c. of a forty-eight-hour-old broth culture was given per os. On April 30th, 250 c.c. of a forty-eight-hour-old broth culture was given per os.

The only symptom shown after giving the culture was dullness on each occasion. No rise of temperature was shown.

On May 25th, 5 c.c. of broth culture was inoculated intravenously. Temperature rose to 103·8°F. and then dropped to normal.

On June 15th, 10 c.c. of broth culture was inoculated intravenously. The temperature rose to 104°F. and then dropped to normal.

This foal has shown no ill effects as a result of the feeding or of the intravenous inoculation of the *B. abortivo equinus* and has remained quite healthy up to date (20-10-21).

Donkey stallion (age five years, condition good).—On January 27th, 1921, was inoculated intravenously with 10 c.c. of a forty-eight-hour-old broth culture of *B. abortivo equinus* (D). No symptoms were shown. On February 18th, this donkey went lame with arthritis of the near shoulder joint. The lameness persisted some months but gradually diminished. Eight months after the inoculation it was going almost sound.

Conclusion. The intravenous and alimentary infection of foals with the *B. abortivo equinus* failed to set up symptoms of joint-ill.

The intravenous inoculation of the organism into a five-year-old donkey stallion gave rise to arthritis twenty-one days later.

Conclusions.

1. In an outbreak of specific abortion amongst pony and donkey mares at the Government Farm, Hissar, Punjab, investigation has shown that the causal agent in both species is the *Bacillus abortivo equinus.* (Good and Corbett).

2. The organism is identical in all particulars with that described by European and American observers.

3. The organism is capable of producing abortion in pony and donkey mares by intravenous, alimentary and intravaginal infection.

4. The incubation period following experimental infection by all routes is shorter in pony than in donkey mares. The average period in pony mares is ten days and in donkey mares nineteen days.

5. The serum of infected pony and donkey mares agglutinates the *Bacillus abortivo equinus* of either pony or donkey origin in high dilutions.

6. The organism failed to set up joint-ill in foals either by intravenous or alimentary infection.

7. Three cases (two in pony mares and one in donkey stallion) of arthritis were observed in adult animals as a result of the intravenous inoculation of the organism.

REFERENCES.

1 Good and Corbett .. Investigation of the Etiology of Infectious Abortion of Mares and Jennets in Kentucky. *Journal of Infectious Diseases*, vol. XIII, July 1913, p. 53.

2 Good and Smith .. The production of a Hyperimmune Serum for Infectious Abortion in Mares. *Journal of Infectious Diseases*, vol. XVIII, 1916, p. 397.

3 Meyer and Boerner .. Studies on the Etiology of Epizootic Abortion in Mares. *Journal of Medical Research*, vol. XXIX, p. 325.

4 Dassonville and Riviere .. Contribution a l'etude de l'avortement épizootique des Juments. *Revue Générale de Médicine Vétérinaire*, Tome XXI, March 1913, p. 237.

5 Neffs .. .. .. Abstract in *Revue Générale de Médicine Vétérinaire*, Tome XXX, June 1921, p. 346.

5a Van Saceghem .. .. L'avortement épizootique au Congo Belge. *Annales de Médicine Vétérinaire*, Jan. 1921, p. 14. (Not seen. Abstract in *Revue Générale de Médicine Vétérinaire*, Tome XXX, June 1921, p. 346.)

6 McFadyean and Edwards Contagious Abortion in Mares and Joint-ill in Foals, *Journal of Comparative Pathology and Therapeutics*, vol. XXX, 1917, p. 321.

7 Schofield .. .. The Etiology of Pyæmic Arthritis in Foals. *Journal of Infectious Diseases*, vol. XV, 1914, p. 409.

8 Edwards .. .. Joint-ill in Foals. A survey of recent investigations. *Veterinary Record*, no. 1706, March 1921.

9 Magnusson .. .. Joint-ill in Foals. Etiology. *Journal of Comparative Pathology and Therapeutics*, vol. XXXII, Sept. 1919, p. 143.

10 McFadyean and Edwards .. Joint-ill in Foals. Etiology and Serum Treatment. *Journal of Comparative Pathology and Therapeutics*, vol. XXXII, December 1919, p. 229.

11 Webb .. .. .. Abortion in a Donkey Stud. *Journal of Comparative Pathology and Therapeutics*, vol. XXII, Dec. 1909, p. 289.

12 Branford and Doyle .. Notes on Contagious Abortion in Pony and Donkey Mares. *Pusa Research Institute Bulletin* No. 121 (1921).

13 Holman .. .. The use of decolourized acid fuchsin as an acid indicator in carbohydrate fermentation tests with some remarks on acid production by bacteria. *Journal of Infectious Diseases*, vol. XV, July 1914, p. 227.

14 De Jong .. .. *Centralbl. f. Bakt.*, LXVII, No. 3, 1912, p. 148. (Not seen. Cited by Meyer and Boerner).

15 Seddon .. .. Some observations on the methods of using the Agglutination Test in the diagnosis of the disease in Bovines caused by the Bacillus of Contagious Abortion. *Journal of Comparative Pathology and Therapeutics*, vol. XXVIII, March 1915, p. 20.

16 Reinhardt and Gauss .. *Zeitschr. f. Infektionskrankh. parasit. krankh u. Hyg.* d. Haust., vol. XVI, No. 4, 13th January, 1915, pp. 219–238.
(Abstract in *Journal of Comparative Pathology and Therapeutics*, vol. XXVIII, 1915, p. 172.)

NASAL GRANULOMA IN CATTLE.*

BY

V. KRISHNAMURTI AYYAR, I.V.S.,

Professor of Pathology and Bacteriology, Madras Veterinary College.

[Received for publication on 7th March, 1924.]

THERE exists in the Madras Presidency a disease condition of common occurrence among cattle which is known as "Nasal Granuloma," or popularly, "Snoring Disease." From information gathered concerning the prevalence of the disease it would appear to cause considerable annoyance and often serious trouble to cattle owners. The condition appears to be highly infective, and as there is, so far as I know, no literature on its ætiology or any work connected with its occurrence, I made arrangements in February, 1922, to obtain material for study from animals suffering from this peculiar disease, and, after having made a preliminary investigation, I contributed a short article upon it to *The Madras Veterinary Journal* in July, 1922, at the special request of the Chief Superintendent, Civil Veterinary Department, Madras.

Though I have not been able to make a complete study of the disease for want of time, yet I believe it would be opportune to place on record certain observations made upon the disease and formations of a causal significance demonstrable in the lesions.

Distribution. The disease is found to have a wide distribution in the Madras Presidency, and cattle of all breeds and both sexes seem to be affected. It is also reported to be prevalent in certain other provinces in India (Assam and Bombay Presidency). At one time it was supposed that only draught cattle, the nostrils of which were punctated with nose strings, became affected,

* Read before the Second Meeting of Imperial Veterinary Officers held at Calcutta in February, 1923, and slightly modified after discussion at the meeting by Mr. Edwards, Director, Imperial Institute of Veterinary Research, Muktesar. The specimens and slides from which photographs and drawings appended to this paper were made were exhibited by the author to the members at the meeting.

but the disease is seen also in heifers, cows and bulls which do not carry the nose strings, with which the Indian transport driver guides his cattle.

Lesions. The lesions are essentially chronic in development and are localised upon and limited to the nasal mucous membrane. In this location they are found as small rounded growths, each varying in size from that of a large millet seed to that of a big pea. The small growths develop in what seem to be multiple, densely packed formations, which in the aggregate suggest the appearance of masses of granulation tissue. Close examination indicates that the growths originate at or near the junction of the nasal mucous membrane and the skin, and on and around the margins of the perforation through which passes the nose string and thence spread to the surrounding mucosa (Plate I, fig. 1). The mucous membrane becomes considerably thickened and considerable irritation is caused during respiration by the occlusion of the nasal passages. The friction would appear to assist in the spread upwards of the condition and sooner or later a large number of coarse growths develop over a large extent of the membrane. The masses formed by the aggregation of nodules may attain in some cases the size of a small walnut with their base intimately adhering to the nasal mucous membrane. As further enlargement of the masses takes place, necrotic foci begin to make their appearance on the surface of the lesions, and multiple abscesses of very minute size are found also to form in their interior. If a well-developed nodule is examined very closely the suppurative centres can readily be seen on the surface (Plate II). The centres are seen as very small whitish or yellowish-white elevations which on palpation seem to contain interiorly some soft material in the nature of pus. If a nodule is squeezed or punctured with a needle, a minute droplet of yellowish thick pus oozes out and a tiny cavity remains on the surface of the lesion. When the lesions are left untouched there is a tendency for them to break down of their own accord and to be sneezed out by the animal, and a raw varied surface is then seen inside the nostril. It is probable that the spread of infection is caused by the infective material discharged in this manner from the nostril. In young nodules this appearance cannot be seen on their surface, but when they are incised one discovers a number of suppurating foci similar to those seen on the surface of the older nodules and containing pus of the same character and consistence.

If one attempts to dissect out these growths much hæmorrhage accompanies the operation. The dissected growths are greyish-red in colour, and very soft and fragile to the touch. How far into the nasal cavity the growths extend or what structural alteration the nasal fossæ have undergone in advanced cases, I cannot say, as I have not had the opportunity to trephine the nasal

fossæ or to make a post-mortem examination of affected animals that have died, for deaths from the disease are very rare. Hitherto, in the few cases that I have examined clinically the upper limits of the affected membrane could be reached with the finger, and thus it would appear that the growths are limited to the anterior nares and are, for the most part, visible from the exterior. It has, however, been reported to me that cases occur in which the growths are found to extend far inwards into the interior of the nostrils.

Etiology. Of causal significance in the disease is a characteristic " ray-fungus " or " club " akin to the formation well known to occur in the common disease condition of Western countries designated *Actinomycosis bovis*. The peculiar " granule " and " club " formation can be demonstrated quite easily in morbid tissues. Of 19 specimens examined, the granule formations have been detected in fourteen.

Histopathology. When sections of the lesion are examined histologically, they are found to be composed of a newly formed typical connective tissue arranged in the form of numerous follicles (Plate I, fig. 2). In the centre of most of these follicles, a large number of round cells are aggregated and held together in a fibrillary reticulated network and it is within this central core of tissue that the granules, presumed to represent colonies of the causal organism, are discoverable (Plate III, figs. 1 and 2). Within other follicles one often finds a collection of polymorphonuclear leucocytes invading and crowding the interior of the follicle in its entirety. The colonies would, therefore, appear to develop within tissue material consisting of fibroblasts which at first multiply owing to the irritation set up and form fibrous tissue distally from the centre of the reaction. As the disease develops the central core of fibroblasts breaks down, and invasion with polymorphonuclear leucocytes takes place with the resulting formation of a minute abscess cavity around the developing or involutionary mass of parasites. In some other foci of infection one can see only degenerated leucocytes and granular cellular detritus.

As the " colonies " increase in size, their composition can be readily resolved into radially disposed " clubs," and meanwhile some of the newly developed fibroblasts commence to become transformed into a fine interstitial connective tissue which subsequently becomes much fibrillated and finally transformed into fibrous tissue (Plate IV, fig. 1). In one of the specimens examined the " granule " formations were found to have penetrated more deeply, involving even the deeper layers of tissue forming the nasal septum (Plate V, figs. 1 and 2).

Increased formation of fibrous tissue and endarteritis forms a predominant feature throughout any section of morbid tissue examined. It is worthy of

note in this connection that Castellani and Chalmers, in describing the lesions of mycetoma in man, state that the fibrous tissue, by encapsulating the "fungus," prevents its spread, while the endarteritis cuts off the supply of nourishment to it (Plate IV, fig. 2). In short, the histopathology of the nasal granuloma lesion is identically the same as that of the lesions found in all mycetomas.

Staining characters of the "colonies." Both the "clubs" and "granules" do not stain well with ordinary aniline dyes, but with Ziehl-Neelsen's method, as commonly employed in staining tubercle bacilli, one obtains excellent results. They are found to be "acid" and "alcohol-fast," and it may be affirmed that some of the colonies are as acid and alcohol-fast as tubercle bacilli. When stained by Ziehl-Neelsen's method, and counterstained with methylene blue, they stain an intense red colour, while the rest of the tissues are stained blue (Plate VI). With a view to obtaining more precise information in regard to the degree of the so-called "acid-fastness" possessed by the colonies, the methods adopted by Herman and by Much for staining acid-fast organisms were applied and the colonies were found to be acid-fast by these methods also (Plates VII and VIII). They were not stainable by the method of Gram.

Whatever methods are employed for staining the "granules" and "clubs" they can be seen clearly in the lesions as relatively coarse masses of various sizes and shapes. Many of the "granule" masses are readily seen to consist of radiating elements—the so-called "rays" or "clubs"—emanating from what seems to be a kind of basal membrane surrounding a central almost hollow core. Some of the rays are short and stumpy having the appearance of typical Indian clubs, while others are long, delicate, and fringe-like (Plate VI, fig. 1 and Plate IX, fig. 1*a*). There would seem to be within each ray a central filament by means of which it is attached to the membrane surrounding the central cavity. It is not improbable that the appearance of a central cavity is due to the shrinkage of the radiating elements during the fixation of the tissues for histological examination, or to the contraction or elimination of the thin purulent matter in which the granules are embedded (Plate IX, fig. 1*b*). In some granules the central space is not a hollow cavity but is found to be occupied with a granular detritus and pus cells with finely shredded filaments radiating from its centre.

Symptoms of the disease. The earliest sign of the disease that attracts the attention of an observer is the frequent sneezing of the animal which is more marked at work than at rest, accompanied by a thick mucous discharge from the nostrils. If one examines the nostrils at this stage, small eruptions

as described already are seen. As the disease advances the nodules increase in number, and a snoring noise becomes more noticeable and the animal sneezes repeatedly. The mucous discharge then becomes profuse and sanguineous at times. When the growths have commenced to disintegrate and to be discharged, the animal is greatly relieved of its difficulty in breathing and the snoring noise becomes less marked, but the relief is only temporary, for the growths recur and cause a repetition of the symptoms. Reports were forthcoming of animals becoming so debilitated by the disease that they succumbed to it.

Mode of infection. As in the cases of other actinomycotic infections, infection in this disease is presumed to take place through wounds, and in view of the very definite localization of the lesions it may be considered *a priori* that the probable portal of entry of the causal agent is therefore through some abrasion or wound in the nasal mucous membrane, which may be either caused by misuse of nose strings or wounds inflicted by coarse grass during grazing. When animals with such abrasions have access to food or water containing the causal agent, these wounds offer a good channel for its entry into the tissues and having once established itself there it excites a chronic or extremely subacute inflammation and undergoes itself a sort of involutionary metamorphosis accompanied by a tissue reaction resulting in the formation of granulation tissue, and the subsequent changes to which allusion has already been made. That the infection of the mucosa or sub-mucosa takes place in close proximity to the skin is closely borne out by Plate IX(2) which shows the " granular " formations very close to the papillæ of the skin.

In considering the possible sources of natural infection among cattle in the Madras Presidency, where the disease is widespread, it is not unreasonable to assume that either the straw on which the cattle depend for the bulk of their ration may harbour the causal agent in a saprophytic form and thus be a source of infection, or infected watering and feeding troughs may offer a good medium for the growth and development of the causal agent and form very likely a source of infection to cattle that have access to them. Of these alternatives, the latter seems to be the more probable one, inasmuch as numerous instances have been brought to my notice in which the disease was reported to have made its appearance in a herd only when the healthy cattle were allowed to drink and feed along with infected cattle. This explanation, however, requires experimental confirmation.

Prophylaxis. From what we know of the nature of other actinomycotic affections and of the very widespread prevalence of this disease, prevention would consist in advising people of the grave danger of allowing their healthy

animals to have access to feeding and watering troughs which have been made available to infected animals, and, as far as possible, infected animals should be provided with feeding and drinking arrangements quite separate from those of the still healthy ones.

Discussion. Having in mind the nature of the strikingly characteristic lesions found upon the nasal mucous membrane of the affected cattle, one appreciates here immediately that one has to deal with a lesion of a comparable origin to that of the well known condition commonly referred to as actinomycosis in cattle. Nasal granuloma differs, however, from the common bovine actinomycosis in the following striking respects, clinically and pathogenetically :—(1) Situation exclusively on the nasal mucous membrane and never in the tissues of the tongue or jaw ; (2) the superficial formation of the characteristic small nodular growths without deep involvement of tissues.

The condition known as actinomycosis in cattle is now known to represent, in reality, more than one etiological entity, although a feature of all types of the affection is the eminently localized development of the lesion together with the presence of the causal organisms in the form of the very well-known stellate granules, which may reach macroscopic dimensions, in the interior of the lesion and which represent involution forms assumed by small aggregations of the causal agents.

In the early days when this disease was first investigated (Bollinger 1877, Israel 1878, Ponfick 1880), the organisms cultivable from the lesions and taken to represent the causal agents of the disease were members of the genus Actinomyces, as now designated according to the most recent system of classification adopted by the Committee of the American Society of Bacteriologists. The organisms included within this genus vary in certain respects among themselves, but possess the common characteristic of forming branching radiating threads, with club-shaped elements as " involution " structures within the lesions set up by them. A striking departure had to be made from the view that the disease was caused by a member of this genus only when Lignières and Spitz (1902) published their observations upon the common causation of actinomycosis in cattle in Argentina by an organism differing considerably, morphologically and culturally, from members of the genus Actinomyces. The organism incriminated by them is a small bacillus 0·4μ in thickness by 1·0 to 1·5μ in length, Gram-negative, non-motile, and designated by them as " Actinobacillus."

Subsequent researches, notably those of Griffith, Bosworth, and of Magnusson have indicated that bovine actinomycosis in Europe is caused probably more frequently by the Actinobacillus than by Actinomyces.

The appearance of "clubs" and "granules" presented by the two etiologically different conditions represented by *Actinomycosis bovis* in lesions is identical. Also, the detailed study of the properties of the two organisms have resulted in their establishment by recent writers within the same bacteriological (or, rather, mycological) group, which would seem to contain organisms of greatly varying characteristics, namely, Family Actinomycetaceæ Buchanan (American Society of Bacteriologists) or Family Nocardiaceæ (Castellani and Chalmers). The American Society of Bacteriologists divide the family Actinomycetaceæ into four genera, in which are included Actinobacillus (containing the single species *Actinobacillus lignièresi* only) and Actinomyces (with *Actinomyces bovis* Harz as the type species and comprising 64 species in all). Castellani and Chalmers include both organisms in the genus Nocardia Toni and Trevisan, 1889, of their family: *Nocardia bovis* (Harz, 1877) belongs to Section 2, *Parasitica* Foulerton, 1910 (Subsection 2, *Majora*), and *Nocardia lignièresi* (Brumpt, 1910) to Subsection 3, *Brevis*, of the same section.

It is beyond the scope of this paper to discuss further the affinities of the parasites responsible for the well known bovine affection *Actinomycosis bovis* which, curiously, so far as I am aware, is unknown or, at least, has not been recorded in India. It would seem that the causal agent of "nasal granuloma" must be closely allied to those of actinomycosis, that is, to organisms classified as Actinomycetaceæ, Nocardia, or, under what is now generally regarded as merely a descriptive term when applied to such organisms, "Streptothrix." Further laboratory investigations are necessary to elucidate this point, but I regret that through want of time I have not been able to undertake them.

Conclusions.

1. There exists a widespread disease condition among cattle in the Madras Presidency and some other parts of India, characterized by the development of chronic granulomatous formations upon the nasal mucous membrane.

2. The disease appears to be of an infective origin.

3. Clinically, the disease is manifested by a snoring noise due to the obstruction of the nasal passages and this symptom is aggravated by any condition which causes increased respiration. The disease is seldom fatal.

4. Histologically, the lesion resembles closely that of *Actinomycosis bovis*, and in the tissue follicles composed of connective tissue elements, one can readily demonstrate granules very similar in appearance and texture to the so-called "ray-fungus" of actinomycosis.

I am very much indebted to Mr. Aitchison, Principal of the Madras Veterinary College, for the facilities he afforded me in carrying out this investigation, and I take the opportunity of conveying to him my grateful thanks for his sympathetic help.

REFERENCES.

(1) CASTELLANI and CHALMERS. *Manual of Tropical Medicine*, Third Edition, 1919.

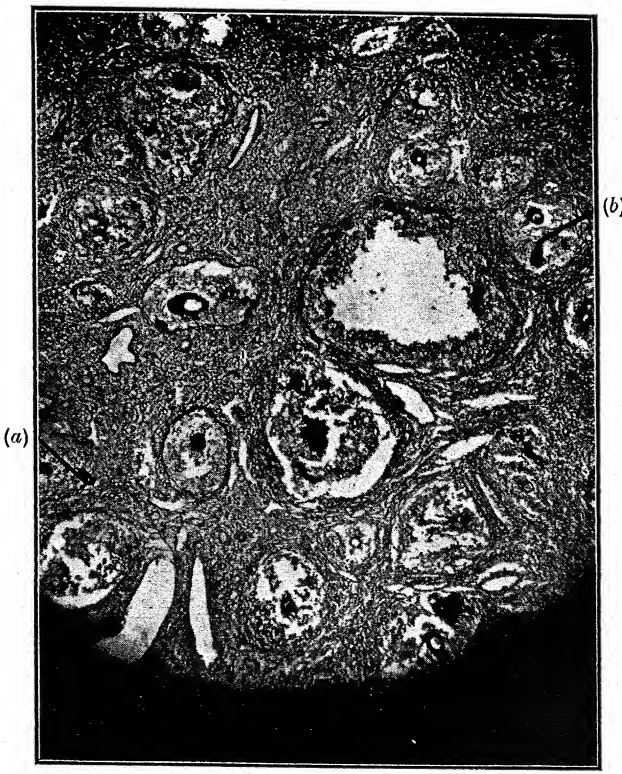
(2) GRIFFITH, F. A Preliminary Report on the Pathology of Bovine Actinomycosis. *Reports to Local Government Board*, New Series, 1915, No. 107.

(3) BOSWORTH, T. J. The Causal Organisms of Bovine Actinomycosis. *The Journal of Comparative Pathology and Therapeutics*, March 1923, Vol. 36, No. 1, pp. 1–22.

(4) MAGNUSSON, H. Experimental Actinomycosis in Cattle. *Revue Generale de Medicine Veterinaire*, 1923, Tome XXXII, p. 613.

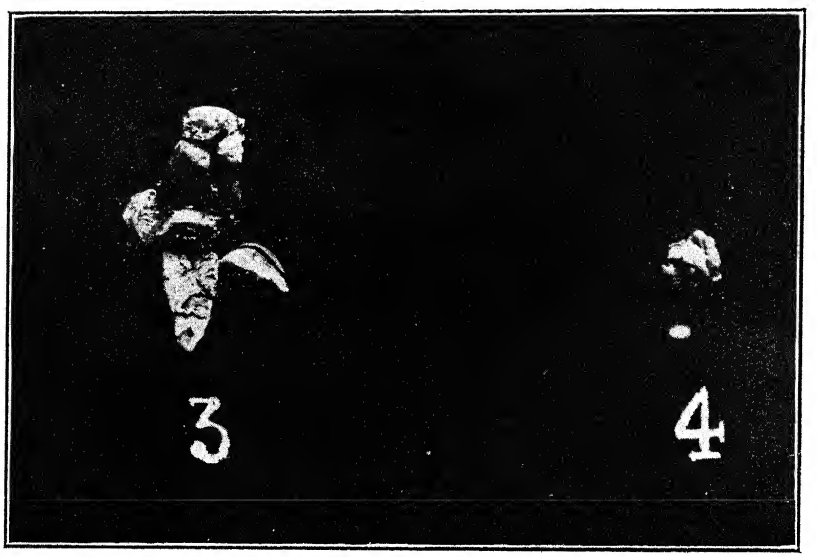
(5) BERGEY'S *Manual of Determinative Bacteriology*. A Key for the Identification of Organisms of the Class Schizomycetes. Arranged by a Committee of the Society of American Bacteriologists. 1923 : Baltimore : Williams & Wilkins Company.

1. Nostril of a bullock suffering from Nasal Granuloma. (Growths are seen in and around the nose strings as white specks.)



2. Section of nasal growth from a bullock suffering from Nasal Granuloma. (*a*) Follicles. (*b*) Granules in different formations occupying the centre of the follicles.

PLATE I.



Growths of suppurative foci and nodular formations removed from a bullock suffering from Nasal Granuloma. (1, Growth with a number of nodules aggregated in masses; 2 and 3 show necrotic foci and multiple abscesses of minute size in the interior, on section seen as white specks; 4 shows suppurative areas on the surface of the nodules.)

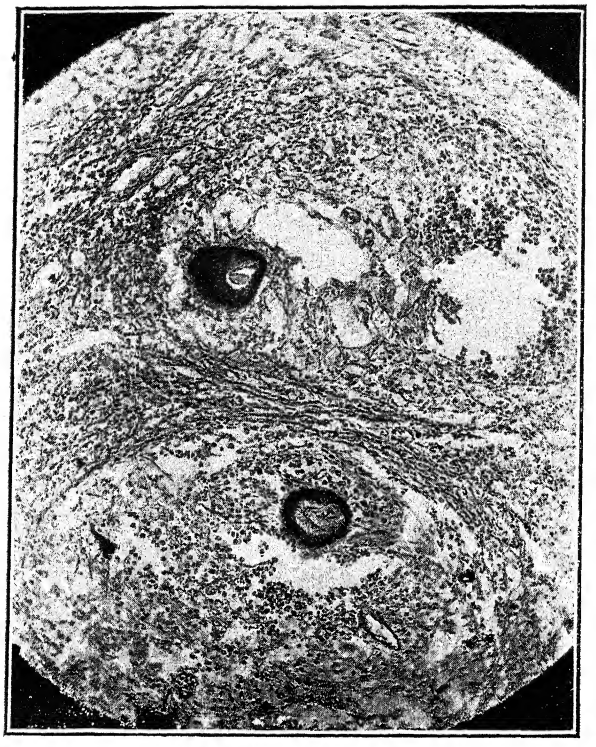


FIG. 1 (× 140).

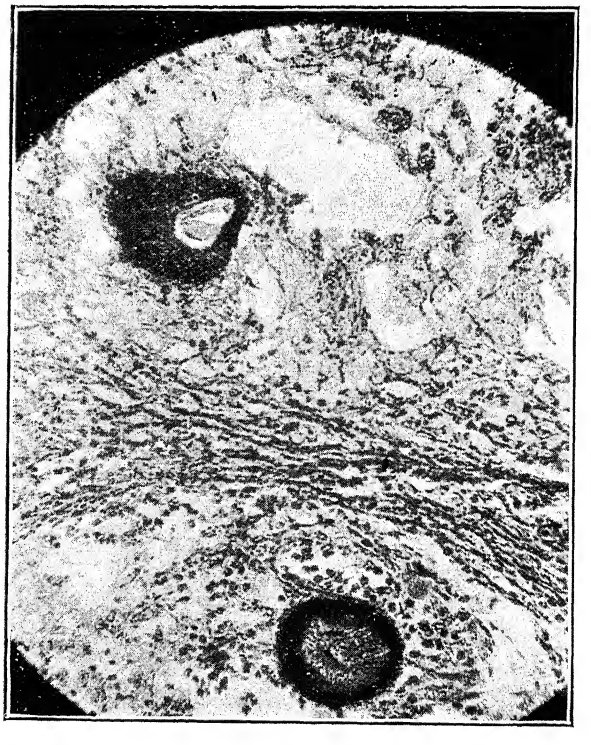
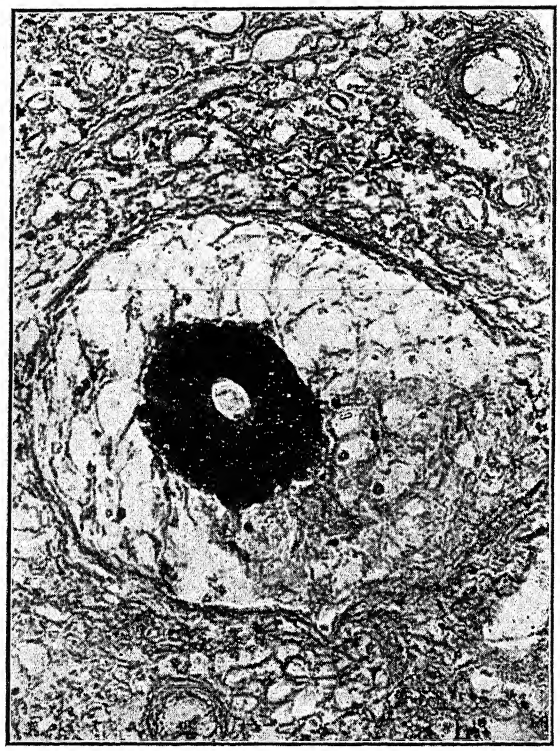
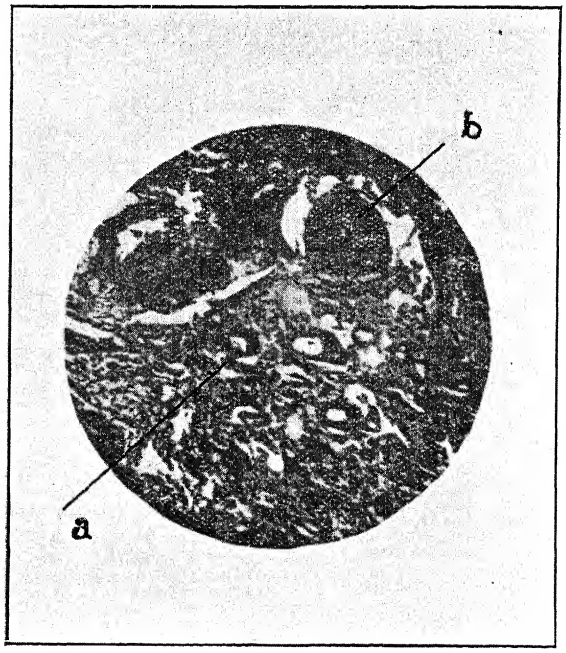


FIG. 2 (× 260).

Section of nasal growth from a bullock. (Round cells and formation of fibrillar reticulated substance clearly seen round the granules.)



1. Section of nasal growth from a bullock (× 300). (A granule highly magnified.)



2. Section of nasal growth from a bullock suffering from Nasal Granuloma (× 15). (*a*) Increased formation of fibrous tissue. (*b*) Endarteritis obliterans.

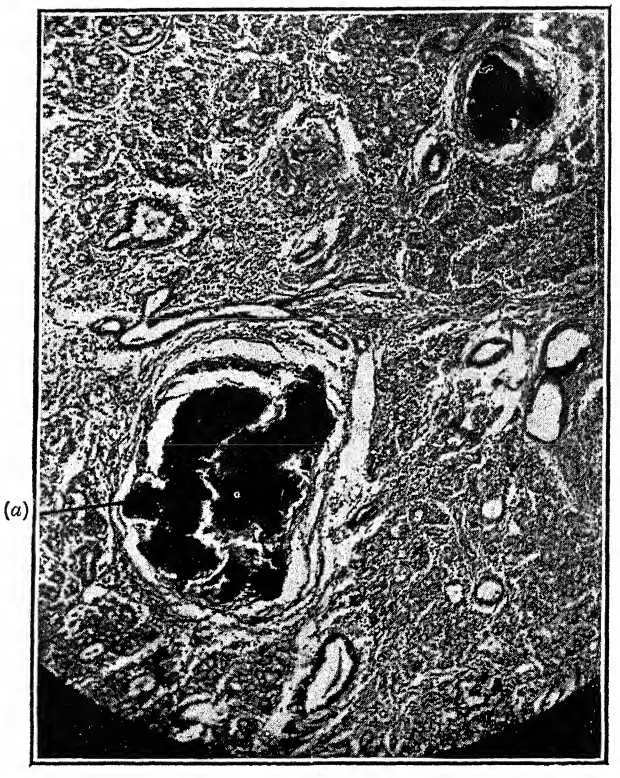


FIG. 1 (× 70).

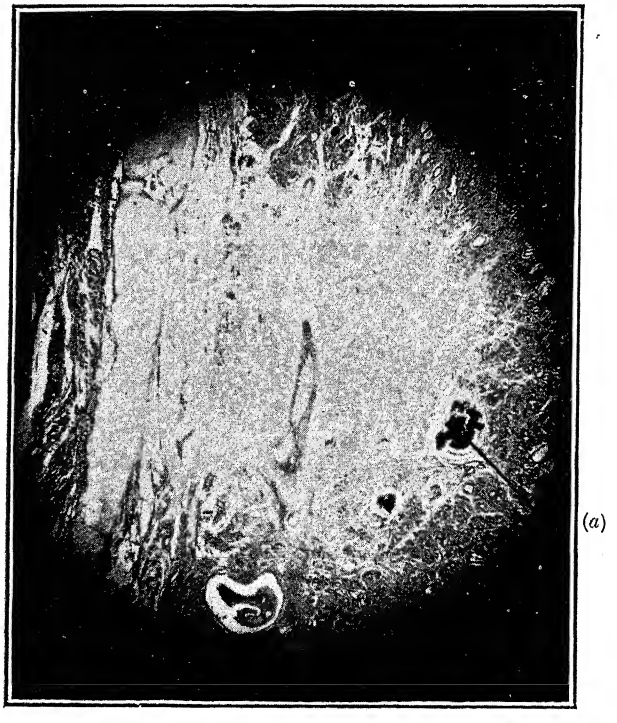
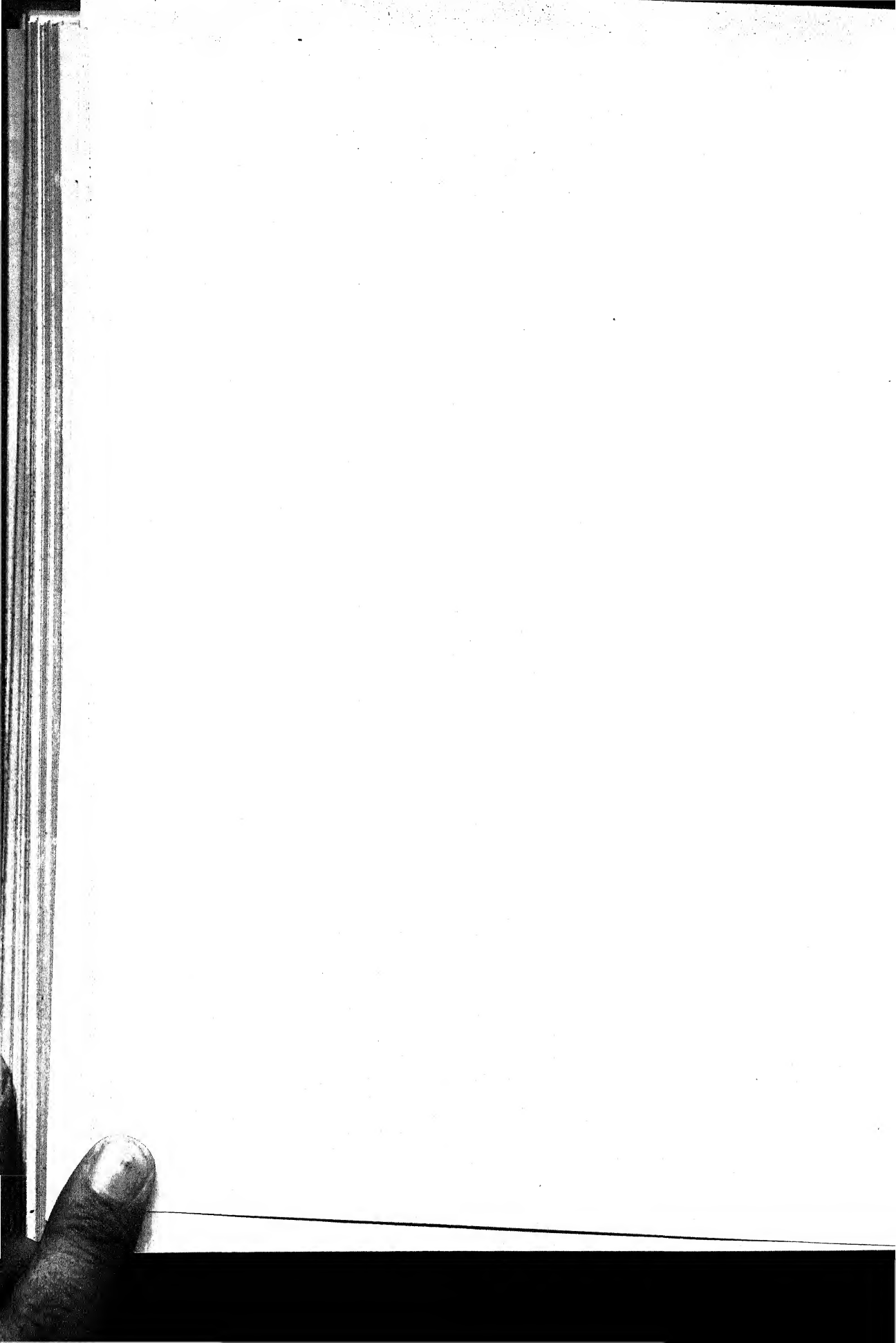
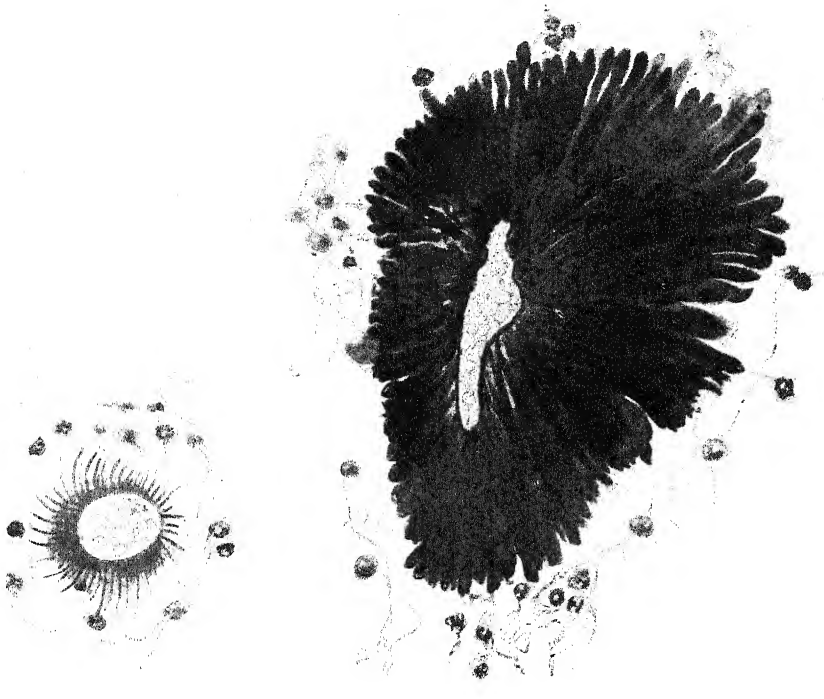


FIG. 2 (× 15).
Section of nasal growth from a bullock. (*a*) The granular formations penetrating deep into mucous glands.



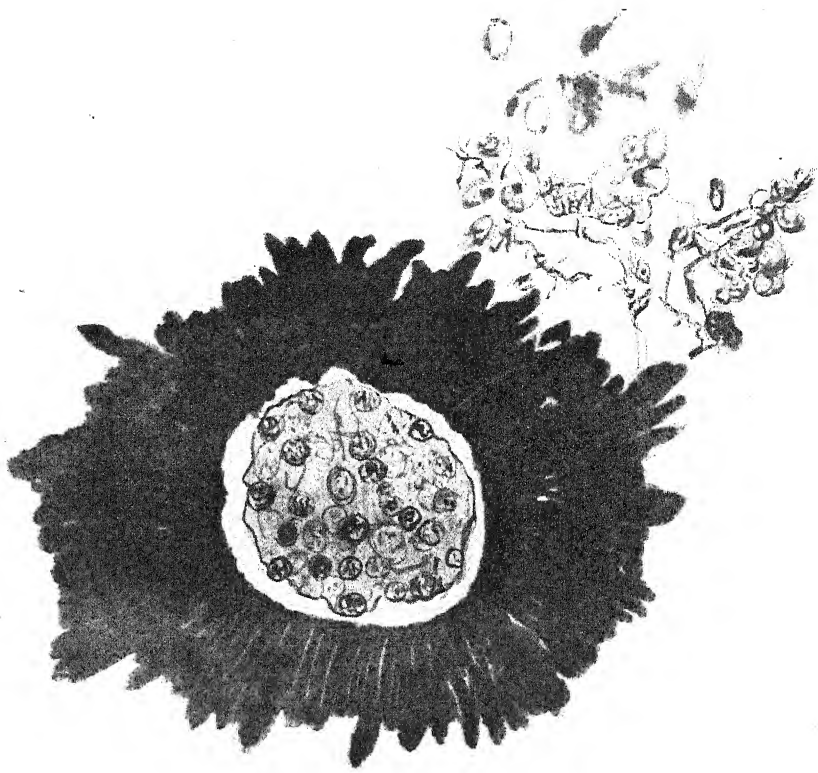


From a section of nasal growth from a bullock.

Fig. 1. Granule showing long and delicate fringe-like filaments.

Fig. 2. Granule showing clubs radiating from the central clear space.

Drawn to scale with camera lucida, $\frac{1}{6}$th objective, No. 4 eye-piece. Stained by Ziehl-Neelsen's method.

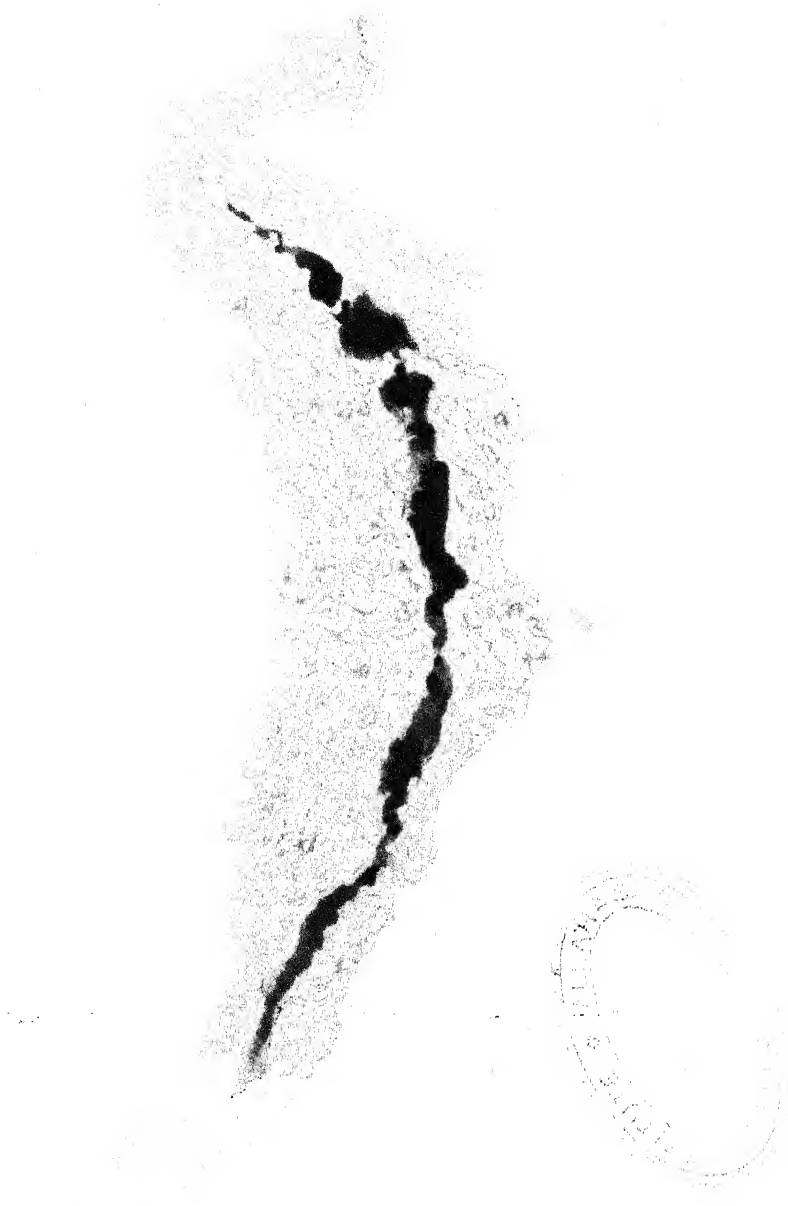


0·05 mm.

From a section of nasal growth from a bullock.

Granule showing clubs radially arranged.

Drawn to scale with camera lucida, $\frac{1}{6}$th objective, No. 2 eye-piece. Stained by Much's method.

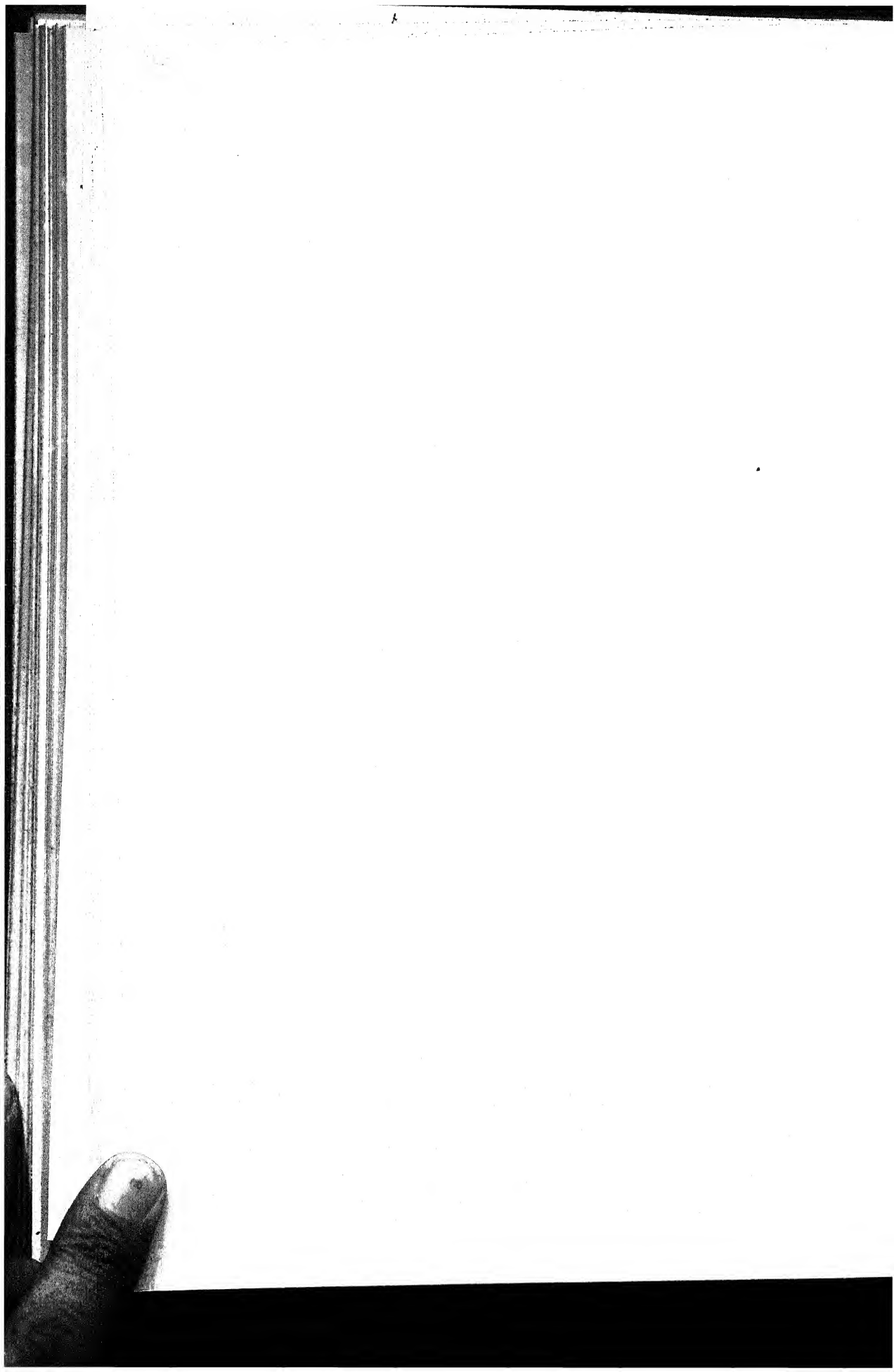


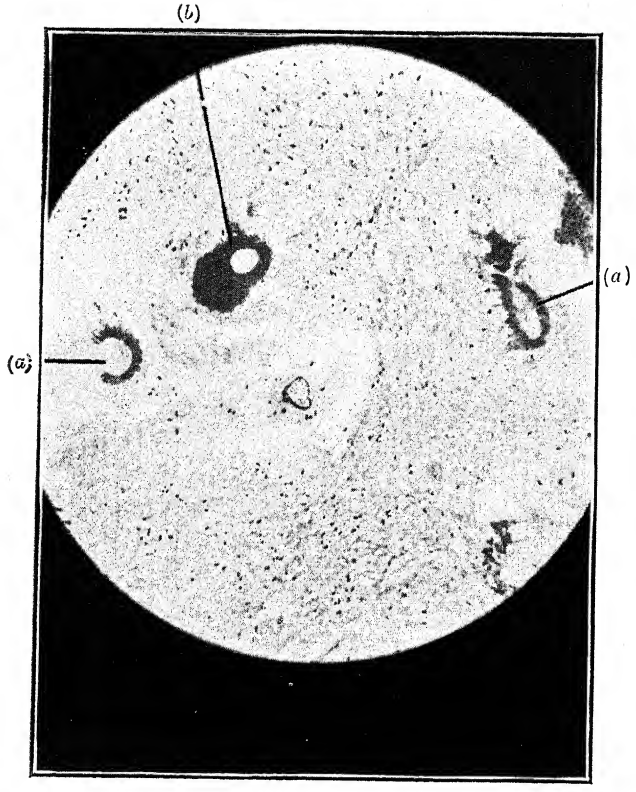
0·05 mm.

From a section of nasal growth from a bullock

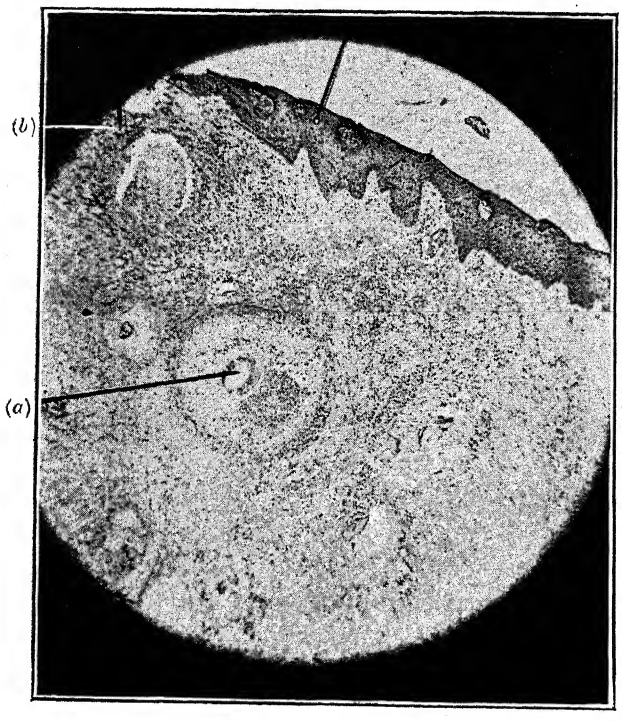
Granule arranged along the margin of the follicle.

Drawn to scale with camera lucida, $\frac{1}{6}$th objective, No. 4 eye-piece. Stained by Herman's method.





1. (*a*) Granule showing long and delicate filaments.
   (*b*) The central cavity in the granule due probably to the shrinkage of the radiating elements during fixation of the tissues.



2. (*a*) Granule occupying the centre of a follicle.
   (*b*) Focus of proliferative irritation indicative of the

CONTENTS

EXPERIMENTS ON THE TREATMENT OF HOOKWORM INFECTION IN DOGS.

BY

AMARNATH GULATI, M.Sc. (PUNJAB),

Imperial Institute of Veterinary Research, Muktesar.

(Received for publication on 9th March 1926.)

1. Introduction.

Ankylostomiasis in dogs has been known by more than one name, such as "Pernicious anæmia," "Bleeding from the nose," "Uncinariosis," and "Hæmorrhagic Enteritis," etc. Packs of hounds suffer seriously from its ill effects, and the disease has proved to be a serious impediment in the breeding of sporting and other pure-bred dogs.

The disease, as it occurs in dogs, is not analogous to the human affection, although the species concerned in the causation of the disease in the two cases are closely allied. As Nicoll (1912) points out, the chronic nature of the disease in man, characterized by progressive anæmia gradually proceeding to a fatal issue is a contrast to the very acute and rapidly fatal disease of dogs.

It is interesting to note here the views of early workers in regard to the nature of the disease in dogs.

Thiroux and Teppay (1907) confused the disease with malaria.

Cuille (1908) describes the disease as an acute dysenteric enteritis.

Conreur and Urbain (1916) mention a disease of young dogs due to ***Uncinaria stenocephala*** as "Hæmorrhagic Enteritis," which they distinguish from "Pernicious anæmia" as follows :—

"The disease is not to be confounded with pernicious anæmia caused by *Ankylostoma tubaeforme* which occurs in older dogs, or with the epizootic gastro-enteritis of Stuttgart. Treatment with vermifuges has very little effect owing to the acuteness of symptoms."

Allen (1924) and others think that the symptoms are somewhat similar to those seen in man.

Other workers describe it at length and note three different stages ; acute, chronic, and carrier.

The observations recorded in this paper were made on dogs which represented the carrier stage only, and showed no symptoms at all, and were apparently healthy, presumably because there arose no opportunity to extend observations to young pups also. The only pup brought to the writer's notice exhibited no other symptom except stunted growth on account of the presence of hookworms, for it is now reported to be growing well after receiving two treatments.

Since Lane's (1913) early discovery of *Ankylostoma braziliense* as a parasite of both man and dog in India, there has been an increasing realization of the importance of dogs as carriers of this form of infection. From the observations recorded during recent years it would appear, however, that this species and certain other hookworms* infecting man, although parasitic also in dogs, are of relatively infrequent occurrence so that the suspected rôle of dog, as a carrier of human hookworm disease is of negligible importance, as compared with its rôle, as a sheep-killer, disseminator of rabies and hydatid disease, besides being a nuisance to man and his animals by defiling streets, houses, stables, sheds, and even food-stuffs.

The present paper records a series of observations on the prevalence and intensity of infection of hookworm disease as it occurs in dogs at Muktesar and also gives the results of experiments carried out with a view to testing the anthelmintic value of certain drugs, the use of which has yielded very encouraging results in the hands of various workers.

The entire work was conducted at Muktesar under the immediate direction and control of Mr. J. T. Edwards, Director, Imperial Institute of Veterinary Research, to whom the writer desires now to express full acknowledgment and gratitude. He has also pleasure to express his grateful thanks to Mr. H. Cooper, Pathologist, for allowing the writer to include in this paper some *post-mortem* records, of collection of worms from dogs made by him.

2. Prevalence of infection.

The factors that count in producing a clinical picture of hookworm disease in dogs are :—(1) The number of worms harboured, (2) their duration in the body of the host, and (3) susceptibility of the host to their toxins. Adult pariah dogs appear to possess naturally a high resistance against the ill effects of these worms, although as Bush (1917) observes, "Practically every dog is loaded with intestinal worms." The maximum number of hookworms observed in a dog was 524, but in spite of this, the dog exhibited no clinical symptom of disease. Debility and emaciation resulted only after a complication with mange, and in such cases it was difficult to decide to what extent either factor contributed towards the development of the symptoms.

* Miyagawa reports *A. duodenale* from a dog in Tokyo, and Stiles mentions having detected *Necator americans* on two occasions from dogs.

The only record available of the extent of prevalence of ankylostomes in dogs in India is by Anantnarayan Rao (1921), who found 45 per cent. infested with ankylostomes out of the total number of dogs examined *post-mortem* during a period of four years. Out of a total of 104 dogs, from which collection of intestinal worms was made carefully at Muktesar, 100 dogs, *i.e.*, 96·2 per cent., were found to harbour ankylostomes in varying proportions, as represented in the following table :—

29 dogs	105 to 524 hookworms.
28 dogs	50 to 105 „
43 dogs	5 to 50 „
4 dogs	nil „

Besides hookworms which all belonged to the species *Ankylostoma caninum* and *A. braziliense*, a few other species of worms were also observed in numbers as noted below :—

Worms	Number of dogs found harbouring the worms	Number of specimens recovered from one individual
1. Tapeworms of the genera *Taenia, Multiceps, Dipylidium* and *Echinococcus.*	54	1 to 224
2. Ascarids (*B. marginata*)	17	1 to 9
3. *Physaloptera* sp.	2	2 and 3
4. *Gnathostomum spinigerum*	1	1

Microscopic examination of the fæces presented evidence of hookworm infection in all dogs examined. Sheather's technique was exclusively employed for detection of worm eggs. Although well adapted for rapid detection of hookworm eggs, Sheather's method did not, however, prove suitable for the purpose of determining the exact number of eggs contained in a given sample in as much as in certain instances hookworm eggs were detected from the sediment in the centrifuge tubes, parti-

cularly when the slide preparations from the surface fluid could not be made at once after centrifugation.

3. Chemotherapy.

Fülleborn (1924), in discussing the present position of chemotherapy in exotic worm infections, sums up by saying that apart from the introduction of carbon tetrachloride for hookworms, advancement in treatment of exotic helminthic affections is practically limited to antimony for schistosomiasis and dracontiasis, and emetin as a reserve for the former of these. Hall and his collaborators have subjected most of the drugs formerly known to possess anthelmintic properties, to what they designate as "critical tests," and after a long series of experiments have been able to establish what they regard as the specific efficacy of drugs like oil of chenopodium against ascarids, and carbon tetrachloride against hookworms. Since the publication of their work, encouraging results have been obtained by other workers with these drugs in the treatment of helminthic infections occurring in both man and animals. Qualitative and quantitative studies undertaken with the object of ascertaining the mode of action; absorption and excretion of the drugs have resulted in the finding that, as originally suggested by Caius and Mhasker and later confirmed by Hall, it is to its chlorine content that carbon tetrachloride owes its anthelmintic properties, whilst the ascaricidal properties of oil of chenopodium are contained in ascaridol which is the distillation product of this drug.

Before the discovery of carbon tetrachloride, chloroform, a compound of the same series, but with a diminished chlorine content as originally recommended by Alessandrini and used in Hermann's mixture, was found by Hall to possess a comparatively high efficacy against hookworms. Since then, other chlorine derivatives of carbon, such as carbon dichloride, carbon trichloride, and tetrachlorethylene, have been tried, and the last-named drug has been found by Hall and Shillinger (1925) to be as effective or slightly more effective against hookworms than carbon tetrachloride.

Caius and Mhasker (1920-1923), who tested the efficacy of a very large number of drugs in connection with the hookworm inquiry in Madras Presidency, concluded that carbon tetrachloride, thymol, betanaphthol, and ascaridol are most effective against hookworms, and possess equal therapeutic values.

The drugs employed in the experiments described in this paper were oil of chenopodium, chloroform, carbon tetrachloride and tetrachlorethylene, either used alone or in combination. For testing the efficacy of the drugs the standard laid down was the relative number of eggs passed in a fixed quantity of fæces before and after treatment. All worms expelled were carefully searched and collected from fæces passed during 24 hours after treatment in all experiments except Experiment I. The reason for not destroying the dogs, as Hall had done, but depending upon egg counts only was to obtain as much information as possible from a limited number of animals.

Details of Experiments and Discussion of Results.*

Experiment I.

Tests of a combination of oil of chenopodium at the rate of 0·1 c.c. per kilogram body weight in soft gelatine capsules and chloroform at the rate of 0·2 c.c. per kilogram body weight mixed with 30 c.c. of castor oil administered simultaneously.

Protocols. Dog No. 4 ; 9 kg. ; starved for 24 hours before each treatment ; 0·9 c.c. of ol. chenopodium administered in soft gelatine capsules ; 30 c.c. of castor oil thoroughly mixed with 1·8 c.c. of chloroform drenched simultaneously ; treated thrice, on 15. II. 25, 5. III. 25 and 13. IV. 25 respectively. No symptoms followed.

Dog No. 5 ; 13. 2 kg. ; starved for 24 hours before each treatment ; 1·3 c.c. of ol. chenopodium in soft gelatine capsules ; 30 c.c. of castor oil thoroughly mixed with 2·6 c.c. of chloroform drenched simultaneously ; treated four times on 5. II. 25, 5. III. 25, 15. III. 25 and 13. IV. 25 respectively. Administration of drug on each of the first two occasions was followed by vomitting ; no other symptoms observed.

Dog No. 6 ; 9·5. kg. ; starved for 24 hours before each treatment ; 0·95 c.c. of ol. Chenopodium in soft gelatine capsules ; 30 c.c. of castor oil thoroughly mixed with 1·9 c.c. of chloroform drenched simultaneously : treated once on 3. IV. 25. No symptoms.

Dog No. 7 ; 9 kg. ; starved for 24 hours before each treatment ; 0·9 c.c. of ol. chenopodium in soft gelatine capsules ; 30 c.c. of castor oil thoroughly mixed with 1·8 c.c. of chloroform drenched simultaneously ; treated once on 3. IV. 25. No symptoms.

Dog No. 13 ; 9 kg. ; starved for 24 hours before treatment ; 0·9 c.c. of ol. chenopodium in soft gelatine capsules ; 30 c.c. of castor oil thoroughly mixed with 1·8 c.c. of chloroform drenched simultaneously ; treated twice on 19. III. 25 and 13. IV. 25. No symptoms.

Dog No. 24 ; 12 kg. ; starved for 24 hours before each treatment ; 1·2 c.c. of ol. chenopodium in soft gelatine capsules ; 30 c.c. of castor oil thoroughly mixed with 2·4 c.c. of choloroform drenched simultaneously ; treated once on 3. IV. 25. No symptoms.

Dog No. 25 ; 12 kg. ; starved for 24 hours before each treatment ; 1·2 c.c. of ol. chenopodium in soft gelatine capsules ; 30 c.c. of castor oil thoroughly mixed with 2·4 c.c. of chloroform drenched simultaneously ; treated thrice on 5. III. 25, 19. III. 25 and 13. IV. 25 respectively. Animal vomitted after second treatment but no other symptoms observed.

---

* The results of egg counts are shown in table appended at the end of the paper.

Dog No. 26; 12·2 kg.; starved for 24 hours before treatment; 1·2 c.c. of ol. chenopodium in soft gelatine capsules; 30 c.c. of castor oil thoroughly mixed with 2·4 c.c. of choloroform drenched simultaneously; treated four times on 15. II. 25, 5. III. 25, 15. III. 25 and 13. IV. 25 respectively; Second treatment followed by vomitting after one hour. (Considerable difficulty was experienced in administering capsules to this dog, as it proved refractory and persistently refused to submit to treatment. No other symptoms observed.

Chart I is illustrative of the general character of the results obtained.

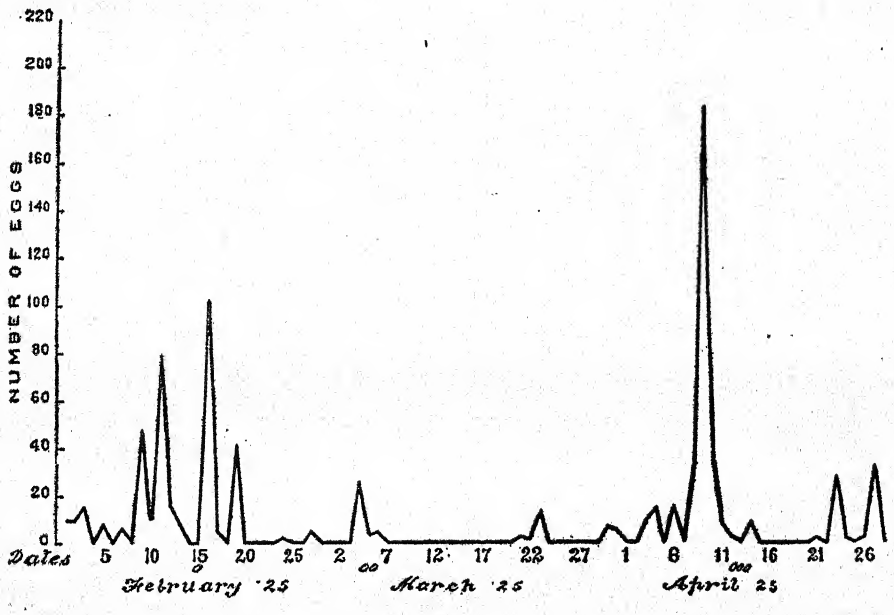


CHART I

Administration of a combination of oil of chenopodium and chloroform. Results of egg counts in the case of dog No. 4.

Discussion. Hall (1919) showed experimentally the efficacy of this combination of ol. chenopodium and chloroform against hookworms of dogs. Both these drugs are also used against hookworm infection in man.

As the worms expelled were not searched for in this experiment, and the dogs were not destroyed after treatment, it is difficult to represent the efficacy in mathematical figures. From the egg counts, as summarised in table, it would appear that the inhibitory action of the treatment on the fecundity of the worms lasted for 7 to 14 days, and in this connexion reference may be made to the results obtained by Mhasker (1924) who observed that ol. chenopodium was more toxic to hookworms

than betanaphthol, thymol, carbon tetrachloride and santonin, for its inhibitory action was evident till the twelfth day after treatment. As the following experiments will show, a more pronounced inhibitory action was exhibited by a combination of ol. chenopodium and chloroform than by a combination of carbon tetrachloride and ol. chenopodium, carbon tetrachloride alone in untreated capsules or even tetrachlorethylene. That the dogs were not "cured" and all the worms were not voided with the fæces is proved by the occurrence of progressively increasing numbers of eggs after the period of inhibition. But as Sawyer (1925) has shown that the destruction of the part of worms harboured in a host increased the multiplication of the remainder, no definite value can be ascribed to the combination employed.

Experiment II.

Tests of a mixture of ol. chenopodium and carbon tetrachloride in the ratio of 1·3 respectively, administered at the rate of 0·3 c.c. per kilogram body weight followed by 30 c.c. of castor oil two hours later, and arecoline hydrobromide at the rate of $\frac{1}{8\frac{1}{2}}$ grain per kilogram body weight drenched two days after the above treatment.

Protocols. Dog No. 4; 9·5 kg.; starved for 24 hours before each treatment; 2·85 c.c. of the mixture of ol. chenopodium and carbon tetrachloride in gelatine capsules; 30 c.c. of castor oil drenched two hours later; 14·5 c.c. of a solution of arecoline hydrobromide prepared by dissolving 1 grain in 50 c.c. of water, drenched two days after; treated once on 15. V. 25. No symptoms. One hookworm and a few tapeworm segments (nearly the whole strobilum without head) were collected on 16. V. 25, from the fæces; no tapeworms expelled after treatment with arecoline hydrobromide.

Pup (red and white, obtained from a private owner); 5 kg.; starved for 24 hours before each treatment; 1·5 c.c. of the mixture in gelatine capsules; 25 c.c. of castor oil drenched two hours later; arecoline hydrobromide not given; treated twice on 3. V. 25 and 11. V. 25, respectively. No symptoms. Three hookworms and two ascarids were collected from fæces on 4. V. 25, *i.e.*, after the first treatment only.

Dog No. 5; 13·2 kg.; starved for 24 hours before each treatment; 3·96 c.c. of the mixture in gelatine capsules; 30 c.c. of castor oil drenched two hours later; 21 c.c. of arecoline hydrobromide solution two days after; treated twice on 16. V. 25 and 17. VI. 25 respectively. No symptoms. No worms expelled with fæces.

Dog No. 6; 9·5 kg.; starved for 24 hours before each treatment; 2·85 c.c. of the mixture in gelatine capsules; 30 c.c. of castor oil two hours later; 14·5 c.c. of arecoline hydrobromide solution two days after; treated once on 8. VI. 25; a capsule got broken in the mouth; symptoms of collapse followed, but raising the hind legs and sprinkling of water brought it back to its senses. No worms expelled with fæces.

Dog No. 7 ; 9 kg. ; starved for 24 hours before each treatment ; 2·7 c.c. of the mixture in gelatine capsules ; 30 c.c. of castor oil two hours later ; 14 c.c. of arecoline hydrobromide solution two days after ; treated thrice on 15. V. 25, 2. VI. 25 and 20. VI. 25 respectively. Treatment with arecoline hydrobromide in the first treatment followed by vomiting ; treatment with castor oil followed by vomiting in the second treatment. No worms expelled with fæces.

Dog No. 13 ; 9 kg. ; starved for 24 hours before each treatment ; 2·7 c.c. of the mixture in gelatine capsules ; 30 c.c. of castor oil two hours later ; 14 c.c. of arecoline hydrobromide solution two days after ; treated on 17. V. 25 and 4. VI. 25 respectively. No symptoms. No worms expelled with fæces.

Dog No. 16 ; 12·2 kg. ; starved for 24 hours before each treatment ; 3·7 c.c. of the mixture in gelatine capsules ; 30 c.c. of castor oil two hours later ; 19 c.c. of arecoline hydrobromide solution two days after ; treated once on 8. VI. 25. No symptoms. No worms expelled with fæces.

Dog No. 21 ; 9 kg. ; starved for 24 hours before each treatment ; 2·7 c.c. of mixture in gelatine capsules ; 30 c.c. of castor oil two hours later ; 14 c.c. of arecoline hydrobromide solution two days after ; treated once on 13. VI. 25. No symptoms. No worms expelled with fæces.

Dog No. 22 ; 12 kg. ; starved for 24 hours before each treatment ; 3·6 c.c of the mixture in gelatine capsules ; 30 c.c. of castor oil two hours later ; 19 c.c. of arecoline hydrobromide solution two days after ; treated twice on 8. VI. 25 and 26. VI. 25 respectively. No symptoms ; the dog died two days after the second treatment. No worms expelled with fæces. 30 hookworms and 1 *Dipylidim* sp. were collected *post mortem*.

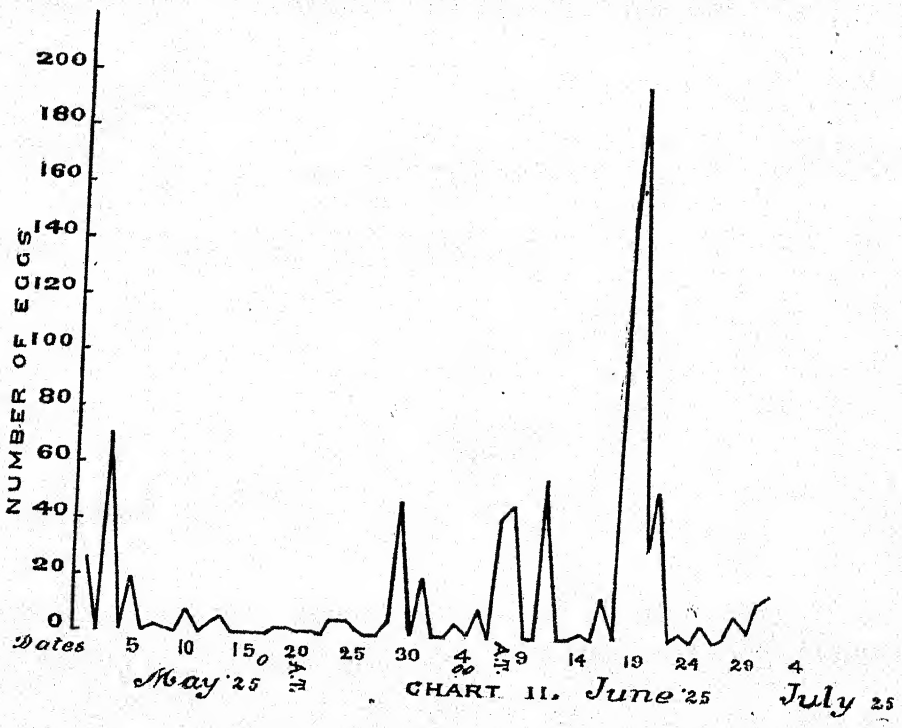
Dog No. 26 ; 12 kg. ; starved for 24 hours before each treatment ; 3·6 c.c. of the mixture in gelatine capsules ; 30 c.c. of castor oil two hours later ; 19 c.c. of arecoline hydrobromide solution two days after ; treated once on 17. VI. 25. No symptoms. No worms expelled with fæces.

Dog No. 336 ; 13·2 kg.; starved for 24 hours before each treatment ; 3·96 c.c. of the mixture in gelatine capsules ; 30 c.c. of castor oil two hours later ; 21 c.c. of arecoline hydrobromide two days after ; treated once on 13. VI. 25. One tape-worm vomited out after the dose of castor oil ; no other symptoms observed. No worms expelled with fæces.

Dog No. 349 ; 15 kg. ; starved for 24 hours before each treatment ; 4·5 c.c. of the mixture in gelatine capsules ; 30 c.c. of castor oil two hours later ; 24 c.c. of arecoline hydrobromide solution two days after ; treated once on 15. VI. 25. No symptoms. 2 hookworms and 4 tapeworms expelled on 16. VI. 25. No tapeworms passed after treatment with arecoline hydrobromide.

Dog No. 429 ; 16 kg. ; starved for 24 hours before each treatment ; 4·8 c.c. of the mixture in gelatine capsules ; 30 c.c. of castor oil two hours later ; 25 c.c. of arecoline hydrobromide solution two days after ; treated once on 11. VI. 25. No symptoms. No worms expelled with fæces.

Chart II is illustrative of the general character of the results obtained.



Administration of a combination of oil of chenopodium and carbon tetrachloride. Results of egg counts in the case of dog No. 13.

Discussion. Hall (1921) found that the combination of carbon tetrachloride and ol. chenopodium at a dose rate of 0·3 c.c. per kilogram body weight was quite effective for removal of hookworms and ascarids from dogs. Later in 1924, he tried a combination of this mixture with arecoline hydrobromide which latter was expected to serve as a teaniacide and a purgative, but the combination was not found to yield satisfactory results in practice. Hall, therefore, suggested the administration of arecoline hydrobromide one or two weeks after treatment with carbon tetrachloride and ol. chenopodium. This interval, however, was considered too long and was therefore reduced to two days only. Even when administered in this manner, the efficacy of both these drugs was found to be considerably minimized.

As would appear from the data, actual worms were expelled in three cases only out of 13 dogs treated, and only one of these three dogs did not show any worm eggs for more than 15 days. Three hookworms and two ascarids were expelled with the fæces in this case, which probably represented all the worms that the dog harboured. Carbon tetrachloride or ol. chenopodium is generally regarded as

valueless against tapeworms, but the results obtained from this experiment would appear to indicate that, as in the case of hookworms, these two drugs exert a certain amount of toxic effect on tapeworms also, for a tapeworm was vomited out in one instance, and in one case 1 tapeworm and in another 4 tapeworms were voided with the excrement. Although arecoline hydrobromide gave poor indications of its action upon tapeworms, nevertheless, when tried alone in a $\frac{1}{4}$ grain dose, on one dog only, 224 tapeworms and 3 hookworms were expelled with the excrement within $\frac{1}{2}$ hour of the administration of the drug. The dog died after two days and was found to harbour two hookworms only, but no tapeworms were present.

Combinations of ol. chenopodium and carbon tetrachloride have been widely tested as to their efficacy in the treatment of human cases. Soper (1924) found that a combination of the two drugs in the proportion of 1:3 was very effective in an area of low ankylostome infection, and like other anthelmintics, this combination gave a high percentage of total worms removed in heavily infested cases, than in those with few worms. In a later paper, Soper (1925) records observations which would appear to indicate, that both these drugs, besides, having species and sex selective action, were more active against *Necator* than *Ankylostoma*.

In view of the highly encouraging results obtained with combination of ol. chenopodium and carbon tetrachloride in the treatment of hookworm infection, as reported by both medical and veterinary workers, there would appear to be little room for doubt, as to the efficacy of these drugs, although their failure in the present experiment might be explained by the fact that the infestation was not heavy, and that the ankylostomes, particularly *A. braziliense*, were of a relatively resistant type. The condition under which Soper (1924) worked would appear to have been more favourable in those respects, since, as remarked by him, "Fortunately the infestation is over 90 per cent. of the less resistant and probably less harmful necator."

Experiment III (*a*).

1. Tests of a single dose of carbon tetrachloride at the rate of 0·3 c.c. per kilogram body weight, and a simultaneous administration of magnesium sulphate.

Protocols. Dog No. 4; 9 kg.; starved for 24 hours before each treatment; 2·7 c.c. of carbon tetrachloride in gelatine capsules; $\frac{1}{2}$ oz. magnesium sulphate drenched simultaneously; treated once on 17. VII. 25. No symptoms. No worms expelled.

Dog No. 5; 13 kg.; starved for 24 hours before each treatment; 3·9 c.c. of carbon tetrachloride in gelatine capsules; $\frac{1}{2}$ oz. magnesium sulphate drenched simultaneously; treated once on 17. VII. 25. No symptoms. No worms expelled.

Dog. No. 13; 9 kg.; starved for 24 hours before each treatment; 2·7 c.c. of carbon tetrachloride in gelatine capsules; $\frac{1}{2}$ oz. magnesium sulphate drenched simultaneously; treated once on 15. VII. 25. Administration of magnesium sulphate followed by vomiting. No worms expelled.

Dog No. 15 ; 9 kg. ; starved for 24 hours before each treatment ; 2·7 c.c. of carbon tetrachloride in gelatine capsules ; ½ oz. magnesium sulphate drenched simultaneously ; treated once on 7. VII. 25 ; the dog was very obstinate in rejecting capsules and only 2 c.c. of the drug could be administered ; profuse salivation followed administration of magnesium sulphate. No worms expelled.

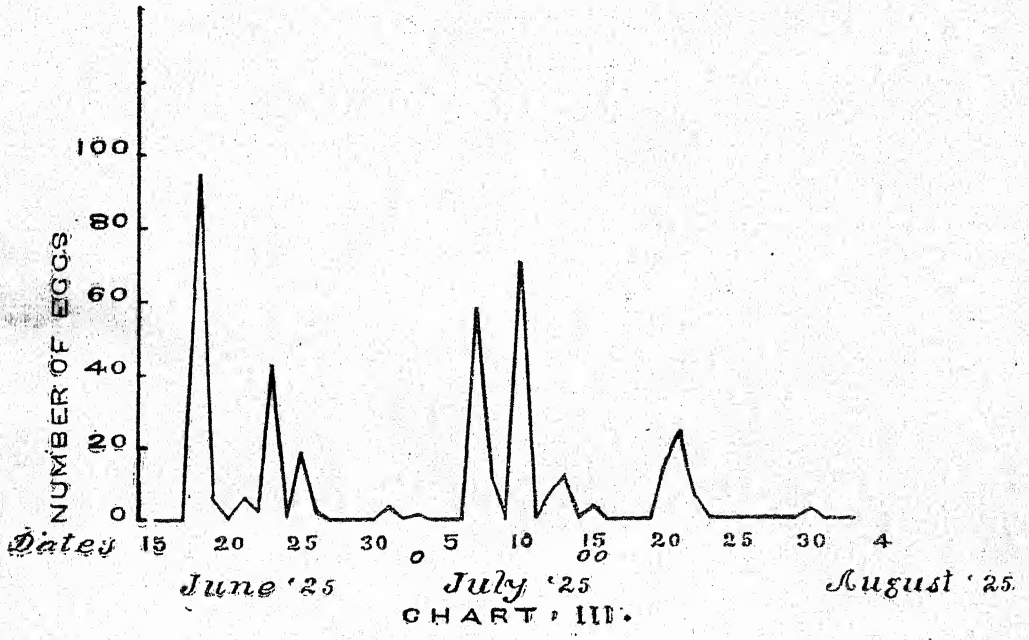
Dog No. 21 ; 9 kg. ; starved for 24 hours before each treatment ; 2·7 c.c. of carbon tetrachloride in gelatine capsules ; ½ oz. magnesium sulphate drenched simultaneously ; treated once on 14. VII. 25. No symptoms. No worms expelled.

Dog No. 25 ; 12 kg. ; starved for 24 hours before each treatment ; 3·6 c.c. of carbon tetrachloride in gelatine capsules ; ½ oz. magnesium sulphate drenched simultaneously ; treated on 3. VII. 25 and 15. VII. 25 respectively ; magnesium sulphate administered with difficulty and the dog attempted to cough out the saline. No worms expelled.

Dog No. 301 ; 12 kg., ; starved for 24 hours before each treatment ; 3·6 c.c. of carbon tetrachloride in gelatine capsules ; ½ oz. magnesium sulphate drenched simultaneously ; treated once on 4. VII. 25 ; magnesium sulphate vomited out ; no other symptoms observed. No worms expelled.

NOTE. The dogs were fed 3 hours after the treatment.

Chart III is illustrative of the general character of results obtained.



Administration of single doses of carbon tetrachloride alone in untreated gelatine capsules. Results of egg counts in the case of dog No. 25.

Discussion. Whether judged by the capacity to dislodge the worms and cause them to be voided with the excrement, or by its inhibitory action on the egg-laying power of female hookworms, carbon tetrachloride gave poor indication of its value in the treatment of ankylostome infestation in dogs at Muktesar. In no instance were worms expelled, and the inhibitory action was seen in two cases only out of seven treated, in one lasting for 4 days and in the other for 6 days.

The fact that hookworms in most cases were found attached to the lower portions of ileum and they occurred very rarely in the duodenum when examined *post mortem* suggested the possibility that the drug failed to reach the worms in sufficient quantities to kill them or even to narcotize them. In view of this possibility the following two procedures were adopted :—

Administration of a double dose of the drug following a prolonged period of starvation to enable the drug to reach the worms.

Administration of a single dose of the drug in keratain-coated capsules to bring the worms within reach of the drug.

2. Tests of double dose of carbon tetrachloride, *i.e.*, 0·6 c.c. per kilogram body weight followed immediately by magnesium sulphate.

Protocols. Dog No. 4 ; 11 kg. ; starved for 48 hours before each treatment ; 6·6 c.c. of carbon tetrachloride in gelatine capsules ; ½ oz. magnesium sulphate drenched simultaneously ; treated twice on 7. XII. 25. and 12.XII.25 respectively. Vomiting followed administration of magnesium sulphate in both treatments and profuse salivation was the only symptom noticeable ; the dog was quite playful and responded to calls ; fed after three hours. No worms expelled.

Dog No. 13 ; 9 kg. ; starved for 24 hours before each treatment ; 5·4 c.c. of carbon tetrachloride in gelatine capsules ; ½ oz. magnesium sulphate drenched simultaneously ; treated once on 11. XII. 25. No symptoms. Fed after three days. No worms expelled.

Chart IV is illustrative of the general character of results obtained.

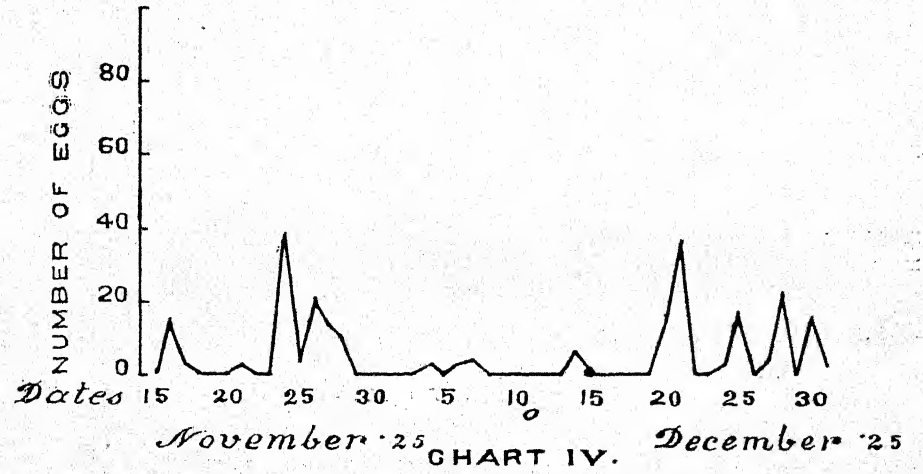


CHART IV.

Administration of double doses of carbon tetrachloride in untreated gelatine capsules. Results of egg counts in the case of dog No. 13.

Discussion. The dose was well tolerated. No increase in the efficacy of the drug was, however, observed when administered in this manner.

Experiment III (b).

Tests on a single dose of carbon tetrachloride administered in keratin-coated capsules, followed immediately by magnesium sulphate.

Protocols. Dog No. 14; 9 kg.; starved for 24 hours before treatment; 3 c.c. of carbon tetrachloride in keratin-coated capsules ½ oz. magnesium sulphate drenched simultaneously; treated once on 25-XII-25. No symptoms; fed after three hours. No worms expelled.

Dog No. 15; 12 kg.; starved for 24 hours before treatment; 4 c.c. of carbon tetrachloride in keratin-coated capsules; ½ oz. magnesium sulphate drenched simultaneously; treated once on 25-XII-25. No symptoms; fed after three hours. No worms expelled.

Dog No. 25; 16·3 kg.; starved for 24 hours before treatment; 5 c.c. of carbon tetrachloride in keratin-coated capsules; ½ oz. magnesium sulphate drenched simultaneously; treated once on 22-XII-25. No symptoms; fed after three hours. No worms expelled.

Dog No. 324; 10 kg.; starved for 24 hours before treatment; 3 c.c. of carbon tetrachloride in keratin-coated capsules; ½ oz. magnesium sulphate drenched simultaneously; treated once on 20-XII-25. No symptoms; fed after three hours. No worms expelled.

Dog No. 395; 16 kg.; starved for 24 hours before treatment; 5 c.c. of carbon tetrachloride in keratin-coated capsules; ½ oz. magnesium sulphate drenched simultaneously; treated once on 25-XII-25. Vomiting followed 10 minutes after administration of magnesium sulphate; fed after three hours. No worms expelled.

Chart V is illustrative of the general character of results obtained.

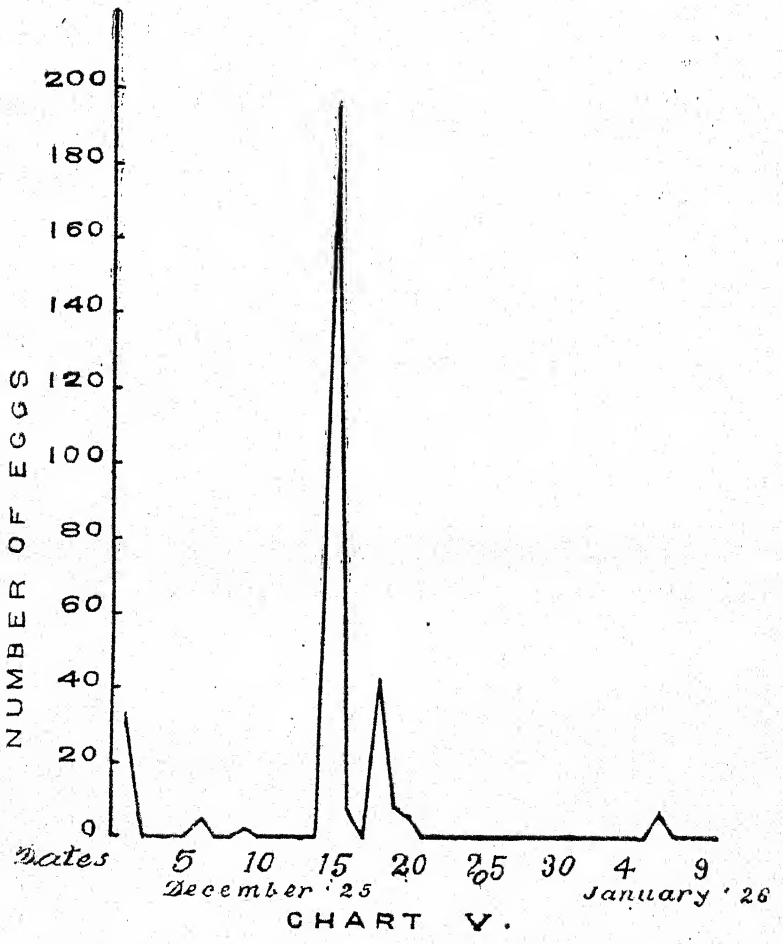


CHART V.

Administration of carbon tetrachloride alone in keratine coated capsules. Results of egg counts in the case of dog No. 15.

Discussion. Caius and Mhasker (1920) found chloroform very effective for removing hookworms from man, and a year later raised the question whether a narcotic less toxic to the host than chloroform would not prove a very efficient remedy for the removal of hookworms. Such a narcotic was found in carbon

tetrachloride when Hall (1921) first demonstrated the value of this drug in the treatment of hookworms of dogs. As a matter of fact, the drug was claimed to be the best anthelmintic then known for hookworms. Allen (1922) found it equally efficacious for hookworms of foxes. Chandler (1924) pointed out that the drug was contra-indicated in the case of hookworm infection in cat. Chandler and Mukherjee (1925) found that carbon tetrachloride was equal or slightly inferior to ol. chenopodium as regards the efficacy in the treatment of *Ankylostoma* infections, although it was distinctly superior in the treatment of *Necator* infections. The failure of the drug, therefore, in the present series of experiments may be partly explained by the absence of *Necator* in dogs at Muktesar. The frequency of *Necator* infestation in man goes to explain partly the success of the drug in human practice. It should be pointed out that as the same dogs were subjected to repeated courses of treatment with these drugs, the possibility just suggests itself that the parasites might have developed a certain measure of resistance to the drugs.

Inhibition on egg laying power of the females was well pronounced in all dogs treated, which shows that a larger amount of drug was brought in contact with the worms when administered in keratin-coated capsules than when given in untreated capsules although actual worms were not passed in any case.

Experiment IV.

Tests of tetrachlorethylene at the rate of 0·3 c.c. per kilogram body weight, not followed by any purgative.

Protocols. Dog No. 4 ; 10 kg. ; starved for 24 hours before treatment ; 3 c.c. of tetrachlorethylene in gelatine capsules ; treated once on 17-1-26. No symptoms. No worms expelled.

Dog No. 5 ; 7·5 kg. ; starved for 24 hours before treatment ; 2·2 c.c. of tetrachlorethylene in gelatine capsules ; treated once on 17-1-26. No symptoms ; died on 18-1-26. No worms expelled. One hookworm collected *post-mortem.*

Dog No. 13 ; 10·4 kg. ; starved for 24 hours before treatment ; 3·2 .c.c. of tetrachlorethylene in gelatine capsules ; treated once on 19-1-26. No symptoms. No worms expelled.

Dog No. 14 ; 9·5 kg. ; starved for 24 hours before treatment ; 3 c.c. of tetrachlorethylene in gelatine capsules ; treated once on 18-1-26. No symptoms. No worms expelled.

Dog No. 21 ; 9 kg. ; starved for 24 hours before treatment ; 2·7 c.c. of tetrachlorethylene in gelatine capsules ; treated once on 19-1-26. No symptoms. No worms expelled.

Dog No. 25 ; 11·5 kg. ; starved for 24 hours before treatment ; 3·2 c.c. of tetrachlorethylene in gelatine capsules ; treated once on 23-1-26. No symptoms. No worms expelled.

Chart VI is illustrative of the general character of results obtained.

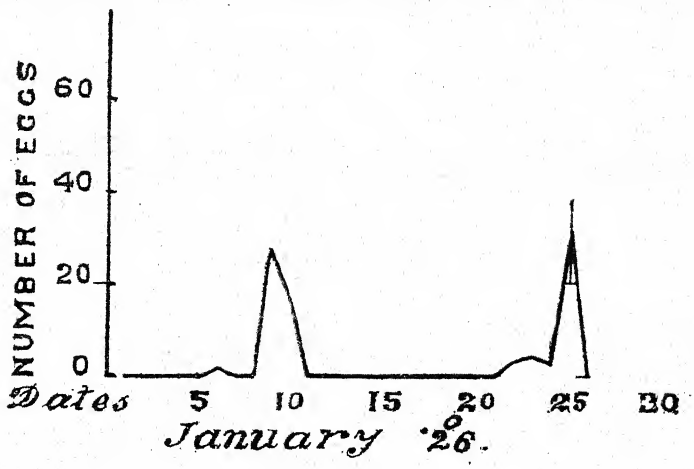


CHART VI.

Administration of tetrachlorethylene. Results of egg counts in the case of dog No. 21.

Discussion. The drug although well tolerated proved to be of little value against hookworm infection of dogs at Muktesar. Actual worms were not voided with the excrement in any case. Instead of an inhibitory action on ovulation as noticed in case of ol. chenopodium and carbon tetrachloride, this drug tended to increase the egg laying power as would appear from the table.

Conclusions.

1. Combinations of ol. chenopodium with chloroform and carbon tetrachloride, carbon tetrachloride alone, and tetrachlorethylene did not prove of value for treatment of hookworm infection in Pariah dogs.

2. A mixture of carbon tetrachloride and ol. chenopodium was found to be the best of all the drugs tested, as it was partially effective in dislodging the worms in three cases.

3. Whether the species of hookworms in question were of a relatively resistant type, or they developed a certain measure of resistance to the drugs on account of the animals being subjected to repeated courses of treatment awaits further investigation. The failure of the drug when administered in keratin-coated capsules, or in double dose with prolonged preceding starvation would appear to indicate that *Ankylostoma braziliense* and *A. caninum* are highly resistant to the effect of carbon tetrachloride.

4. Sawyer (1925) says, " After a thorough treatment of a community for hookworms there was usually an astonishingly rapid rise towards the original amount of

infection, the rate of increase being fastest in the first year after treatment campaign." He therefore suggests that the control measures should be planned on a permanent basis with a view, particularly, to obtaining a reduction of the level of equilibrium of infection in a community, through sanitation, education, and timely repetitions of treatment to secure a progressive diminution of the intensity of infection. On account, however, of the total failure of the drugs tested in these experiments and of the possibility that the parasites develop a certain measure of resistance to the drugs, when repeatedly subjected to a more or less similar course of treatment, a line of investigation that would appear to merit attention is to test the efficacy of the drugs when used in alternation.

REFERENCES.

1. ALLEN (1923). The efficiency of carbon tetrachloride against Hookworms in the Silver Black Fox. *Jl. Amer. Vet. Assoc.*, Vol. 61, p. 31.
2. ALLEN (1924). Ankylostomiasis in a kennel of Airedales. *Vet. Jl.*, Vol. 80, p. 439.
3. ANANTNARAYAN RAO (1921). Ankylostomiasis in dogs. *Madras Vet. Jl.* No. 11, p. 2.
4. ASHFORD (1922). Section on Ankylostomiasis in Byam and Archibald. *The Practice of Medicine in Tropics.*, p. 1848.
5. BUSH (1917). When the dog becomes a nuisance. *Public Health Michigan, N. S.* Vol. 5 (1) pp. 20-22 cited by Hall (1917): Parasites of dog in Michigan. *Jl. Amer. Vet. Med. Asson.*, Vol. 51, p. 383.
6. CAIUS AND MHASKER (1920). The correlation between the chemical composition of anthelmintics and their therapeutic values in connection with the hookworm inquiry in the Madras Presidency, VIII, Chloroform. *Ind. Jl. Med. Res.*, Vol. 8, p. 379.
7. .....(1923). The correlation between the chemical composition of anthelmintics and their therapeutic values in connection with the hookworm inquiry in the Madras Presidency. XXII. Summary and conclusions. *Ind. Jl. Med. Res.*, Vol. 11, p. 371.
8. CHANDLER and MUKERJI (1925). Notes on carbon tetrachloride as an anthelmintic. *Ind. Med. Gaz.*, Vol. 60, p. 61.
9. CONREUR and URBAN (1916). Enterite hemorrhagica doscães novos—Uncinariose intestinal. *Arch. Brasileiros de med.*, Vol. 6, p. 560. (Abs. in *Trop. Vet. Bull.*, Vol. 5, p. 252.)
10. CORT (1925). Investigations on the control of hookworm disease. XXIV. General summary of results. *Amer. Jl. Hyg.*, Vol. 5, p. 49.
11. CUILLE (1908). Acute experimental ankylostomiasis in the dog. *Révue Vétérinaire* No. 10, 1908. (Trans. in *Jl. Trop. Vet. Sci.*, Vol. 4, p. 159).

12. Fülleborn (1924). Referat ueber den jetzigen stand der therapie besonders chemotherapie, bei exotischen wurmkrankheiten. *Arch. Jl. Schiffs. u. Trop. Hyg.*, Vol. 28, p. 526. (Abs. in *Trop. Dis. Bull.*, Vol. 22, p. 457).
13. Hall (1918). Studies on anthelmintics IV. Experiments with combinations of oil of chenopodium and chloroform. *Jl. Amer. Vet. Med. Assoc.*, Vol. 56, p. 59.
14. .....(1921). The use of carbon tetrachloride for the removal of hookworms. *Jl. Amer. Med. Assoc.*, Vol. 77, p. 1641.
15. .....(1924). The effect of magnesium sulphate simultaneously administered, on the efficacy and safety of carbon tetrachloride for the removal of hookworms. *Amer. Jl. Trop. Med.*, Vol. 4, p. 1.
16. .....and Shillinger (1925). Tetrachlorethylene, new anthelmintic. *Amer. Jl. Trop. Med.*, Vol. 5, p. 229.
17. Lane (1913). *Anchylostoma ceylanicum*, a new human parasite. *Ind. Med. Gaz.*, June 1913.
18. Leach (1923). The mode of action of carbon tetrachloride. *Far. Eastern Assoc. Trop. Med. Trans. Fifth Biennial Congress*, Singapore. (Abs. in *Trop. Dis. Bull.*, Vol. 22, p. 457.)
19. Nicoll (1912). The anæmia of ankylostomiasis. *Bnt. Med. Jl.*, Oct. 26, p. 1097.
20. Sawyer (1925). Factors that influence the rate of increase of hookworm infection. *Amer. Jl. Hyg.*, Vol. 5.
21. Schlingman (1925). The effect of carbon tetrachloride on puppies. *Jl. Amer. Vet. Med. Assoc.*, Vol. 67, p. 474.
22. Smillie and Pessoa (1923). Treatment of hookworm disease with carbon tetrachloride. *Amer. Jl. Hyg.*, Vol. 3, p. 35.
23. .....(1925). Treatment of hookworm disease with a mixture of carbon tetrachloride and Ascaridol. *Amer. Jl. Trop. Med.*, Vol. 5, p. 71.
24. Soper (1924). Getting rid of hookworm. *Amer. Jl. Hyg.*, Vol. 4, p. 699.
25. .....(1925). Factors which should determine the selection of an anthelmintic in a geographical area. *Amer. Jl. Hyg.*, Vol. 5, p. 408.
26. Thiroux and Teppay (1907). Ankylostomiasis of the dog in Senegal. *C. R. de la Soc. Biol.*, T. LXI., No. 28. (Trans. in *Jl. Trop. Vet. Sci.*, Vol. II, p. 243.)

MGIPC—IV-2-28—24 12-26—600.

November 1926 Veterinary Series Vol. III, No. 8

Memoirs of the Department of Agriculture in India

On the Occurrence of a Lung Fluke Paragonimus edwardsi, n. sp. in a Palm Civet (Paradoxurus grayi) in Kumaon Hills

BY

AMARNATH GULATI, M.Sc. (Punjab)

Imperial Institute of Veterinary Research, Muktesar

AGRICULTURAL RESEARCH INSTITUTE, PUSA

Calcutta: Government of India
Central Publication Branch
1926

Government of India Publications are obtainable from the Government of India Central Publication Branch, Imperial Secretariat Building, 3, Government Place, W., Calcutta, and from the following Agents :—

EUROPE.

OFFICE OF THE HIGH COMMISSIONER FOR INDIA,
42, GROSVENOR GARDENS, LONDON, S.W. 1.
And at all Booksellers.

INDIA AND CEYLON.

Provincial Book Depôts.

MADRAS :—Office of the Superintendent, Government Press, Mount Road, Madras.
BOMBAY :—Superintendent, Government Book Depôt, Town Hall, Bombay.
SIND :—Library attached to the Office of the Commissioner in Sind, Karachi.
BENGAL :—Office of the Bengal Secretariat Book Depôt, Writers' Buildings, Room No. 1, Ground Floor, Calcutta.
UNITED PROVINCES OF AGRA AND OUDH :—Office of the Superintendent of Government Press, United Provinces of Agra and Oudh, Allahabad.
PUNJAB :—Office of the Superintendent, Government Printing, Punjab, Lahore.
BURMA :—Office of the Superintendent, Government Printing, Burma, Rangoon.
CENTRAL PROVINCES AND BERAR :—Office of the Central Provinces Secretariat, Nagpur.
ASSAM :—Office of the Superintendent, Assam Secretariat Press.
BIHAR AND ORISSA :—Office of the Superintendent, Government Printing, Bihar and Orissa, P. O. Gulzarbagh, Patna.
COORG :—Office of the Chief Commissioner of Coorg, Bangalore.
NORTH-WEST FRONTIER PROVINCE :—Office of the Manager, Government Printing and Stationery, Peshawar.

Thacker, Spink & Co., Calcutta and Simla.
W. Newman & Co., Ltd., Calcutta.
R. Cambray & Co., Calcutta.
S. K. Lahiri & Co., Calcutta.
The Indian School Supply Depôt, 309, Bow Bazar Street, Calcutta, and 226, Nawabpur, Dacca.
Butterworth & Co. (India), Ltd., Calcutta.
Rai M. C. Sarcar Bahadur & Sons, 90-2A, Harrison Road, Calcutta.
The Weldon Library, 17, Park Street, Calcutta.
Standard Literature Company, Limited, Calcutta.
Association Press, Calcutta.
Chukervertty, Chatterjee & Co., Ltd., 13, College Square, Calcutta.
The Book Company, Calcutta.
James, Murray & Co., 12, Government Place, Calcutta. (For Meteorological Publications only.)
Roy Choudhury & Co., 68/5, Russa Road, North, Calcutta.
Higginbotham & Co., Madras.
V. Kalyanarama Iyer & Co., Madras.
P. R. Rama Iyer & Co., Madras.
Rochouse and Sons, Madras.
G. A. Natesan & Co., Publishers, George Town, Madras.
The Modern Stores, Salem, Madras.
Bright & Co., Trivandrum.
The Booklover's Resort, Taikad, Trivandrum, South India.
V. S. Swaminathan, Bookseller, West Tower Street, Madura.
E. M. Gopalakrishna Kone, Pud..mandapam, Madura.
Vijapur & Co., Vizagapatam.
Thacker & Co., Ltd., Bombay.
D. B. Taraporevala, Sons & Co., Bombay.
Sunder Pandurang, Bombay.
Ram Chandra Govind & Sons, Kalbadevi, Bombay.
N. M. Tripathi & Co., Booksellers, Princess Street, Kalbadevi Road, Bombay.
R. B. Umadikar & Co., The Bharat Book Depôt, Dharwar.
Proprietor, New Kitabkhana, Poona.
The Manager, Oriental Book Supplying Agency, 15, Shukrawar, Poona City.
R. S. Gondhalekar's Book Depôt, Publisher and Bookseller, Budhwar Chawk, Poona City.
Managing Director, Co-operative Bookstall, Booksellers and Publishers, Poona City.
The Standard Bookstall, Karachi, Quetta, Delhi, Murree and Rawalpindi.
The Standard Bookstall, Quetta.
J. Ray & Sons, 43 K. & L., Edwardes Road, Rawalpindi and Murree.
The Standard Book Depôt, Lahore, Lucknow, Nainital, Mussoorie, Dalhousie and Ambala Cantonment.
Karsandas Narandas & Sons, Surat.
Mangaldas & Sons, Booksellers and Publishers, Bhaga Talao, Surat.
Mrs. Radhabai Atmaram Sagoon, Kalbadevi Road, Bombay.
A. H. Wheeler & Co., Allahabad, Calcutta and Bombay.
The Karachi Book Depôt, Karachi.
N. B. Mathur, Supdt., Nazir Kanun Hind Press, Allahabad.
The North India Christian Tract and Book Society, 18, Clive Road, Allahabad.
Ram Dayal Agarwala, 184, Katra, Allahabad.
Manager, Newal Kishore Press, Lucknow.
The Upper India Publishing House, Ltd., 41, Aminabad Park, Lucknow.
Munshi Seeta Ram, Managing Proprietor, Indian Army Book Depôt, Juhi, Cawnpore.
Rai Sahib M. Gulab Singh & Sons, Mufid-i-Am Press, Lahore and Allahabad.
Rama Krishna & Sons, Booksellers, Anarkali, Lahore.
Puri Brothers, Booksellers and Publishers, Katcheri Road, Lahore.
The Tilak School Bookshop, Lahore.
The Standard Bookstall, Lahore.
Manager of the Imperial Book Depôt, 63, Chandney Chawk Street, Delhi.
Oxford Book and Stationery Company, Delhi.
Superintendent, American Baptist Mission Press, Rangoon.
Proprietor, Rangoon Times Press, Rangoon.
The Modern Publishing House, Ltd., 30, Phayre Street, Rangoon.
The International Buddhist Book Depôt, Post Box No. 971, Rangoon.
Burma Book Club, Ltd., Rangoon.
Manager, the "Hitavada," Nagpur.
Bhisey Brothers, Booksellers, and Stationers, Sitabaldi, Nagpur.
S. C. Talukdar, Proprietor, Students & Co., Cooch Behar.
Times of Ceylon Co., Ltd.
The Manager, Ceylon Observer, Colombo.
The Manager, The Indian Book Shop, Benares City.
B. C. Basak, Esq., Proprietor, Albert Library, Dacca.
The Srivilliputtur Co-operative Trading Union, Ltd., Srivilliputtur (Satur S. I. R.).
Banwari Lall, Esq., Pakariya Street, Pilibhit, United Provinces.
The Manager, Educational Book Depôt, Jubbulpore.
Raghunath, Prasad & Sons, Patna City.
Dandekar Brothers, Indore City.
The Hyderabad Book Depôt, Chaderghat, Hyderabad, Deccan.

ON THE OCCURRENCE OF A LUNG FLUKE *PARAGONIMUS EDWARDSI*, N. SP., IN A PALM CIVET (*PARADOXURUS GRAYI*) IN KUMAON HILLS.

BY

AMARNATH GULATI, M.Sc. (PUNJAB)

Imperial Institute of Veterinary Research, Muktesar.

(Received for publication on 17th February 1926.)

Cobbold (1859) recorded for the first time from India the occurrence of a lung fluke from an ichneumon (Herpestes mungo), and named it *Distoma compactum*. In 1899, Stiles and Hassal revised the name of this species in accordance with the rules of zoological nomenclature and placed it in the genus *Paragonimus*, under the name of *P. compactus* (Cobbold, 1859).

Since this fluke was originally discovered and described, no further report of its occurrence in India would appear to have been published till 1923, and Ward and Hirsch (1915), in the course of a comparative study of the various species of *Paragonimus*, refer to the paucity of information relating to *P. compactus*. Surveyor (1919) detected the presence of eggs, believed by him to be those of *Paragonimus*, from the faeces and sputum of a Chinaman in India, but there is no indication as to the species of *Paragonimus* dealt with by him.

Lane and Low (1922) mention a sporadic distribution of *Paragonimiasis* in India, but do not state what species have been actually recorded.

Vevers (1923) records the occurrence of *P. compactus*, *P. westermanii*, *P. kellicotti* from India. *P. compactus* has been redescribed by him, and in arriving at the identity of this species Vevers has been guided mainly by a consideration of the morphological peculiarities presented by the cuticular spines, in accordance with the method initiated by Ward and Hirsch in the course of their classificatory work relative to other species of *Paragonimus*. In the literature at his disposal, the writer does not find any reference to the hosts from which Vevers made his collections. The recorded hosts of *Paragonimus* comprise the following species of animals, tiger, cat, dog, hog, a Brazilian otter, an Indian ichneumon, and man.

The present paper deals with what appears to be an unrecorded species of *Paragonimus* from apparently a new species of mammalian host (*Paradoxurus grayi*). In all eight cysts were discovered in the substance of the lungs. An incision made into each one of these cysts liberated a pair of flukes lying together. Sixteen specimens thus collected were subjected to microscopical examination and the following description was worked out.

Paragonimus edwardsi, n. sp.

External Characters. The body is thick and plump ; pinkish to reddish-brown in colour ; more or less spindle-shaped, with attenuated extremities. The anterior end tends to bend towards the ventral side. The ventral surface is flattened, the dorsal arched and convex. Transverse section is nearly semicircular.

Measurements of fifteen specimens are shown in the following table :—

	Length	Breadth	Thickness	Remarks
	mm.	mm.	mm.	
1	4·6	1·7	0·5	Compressed.
2	8·0	4·0	0·7	Do.
3	13·0	4·2	3·0	Not compreessd.
4	12·0	4·1	2·7	Ditto.
5	11·0	5·0	3·2	Ditto.
6	11·6	4·5	3·5	Ditto.
7	12·0	3·5	2·8	Ditto.
8	12·2	5·0	3·0	Ditto.
9	12·7	3·2	2·5	Ditto.
10	10·3	3·5	2·3	Ditto.
11	10·0	4·0	2·7	Ditto.
12	11·0	3·2	3·0	Ditto.
13	9·8	3·2	3·3	Ditto.
14	10·0	3·3	2·7	Ditto.
15	11·4	4·3	3·2	Ditto.
Average	10·64	3·78	2·6	Ditto.

The oral sucker is terminal, and is slightly smaller than the ventral sucker. The latter is situated at about one third of the whole length from the anterior extremity ; it appears as a circular pit on the ventral surface. The cuticular spines are irregular in outline, more or less uniform in size all over the body, and are unlike those of *P. compactus* as described by Vevers (1923) ; nor do they conform to any types figured by Ward and Hirsch (1915). In addition to the spines the cuticle also shows scale-like wrinklings. Internal anatomy :—

Digestive system. The oral sucker opens into a sharply defined and circular pharynx, which leads into a short and delicate oesophagus. The oesophagus

EXPLANATION OF PLATE X.

FIG. A. Camera lucida drawing of a mounted specimen of *Paragonimus edwardsi*. Ventral view. (× 11.)

Do. B. Diagrammatic sketch of a cyst showing a pair of flukes seen on making an incision into it. (× 3.)

Do. C. Diagrammatic sketch of the fluke. Naked eye appearance, ventral view. (× 3.)

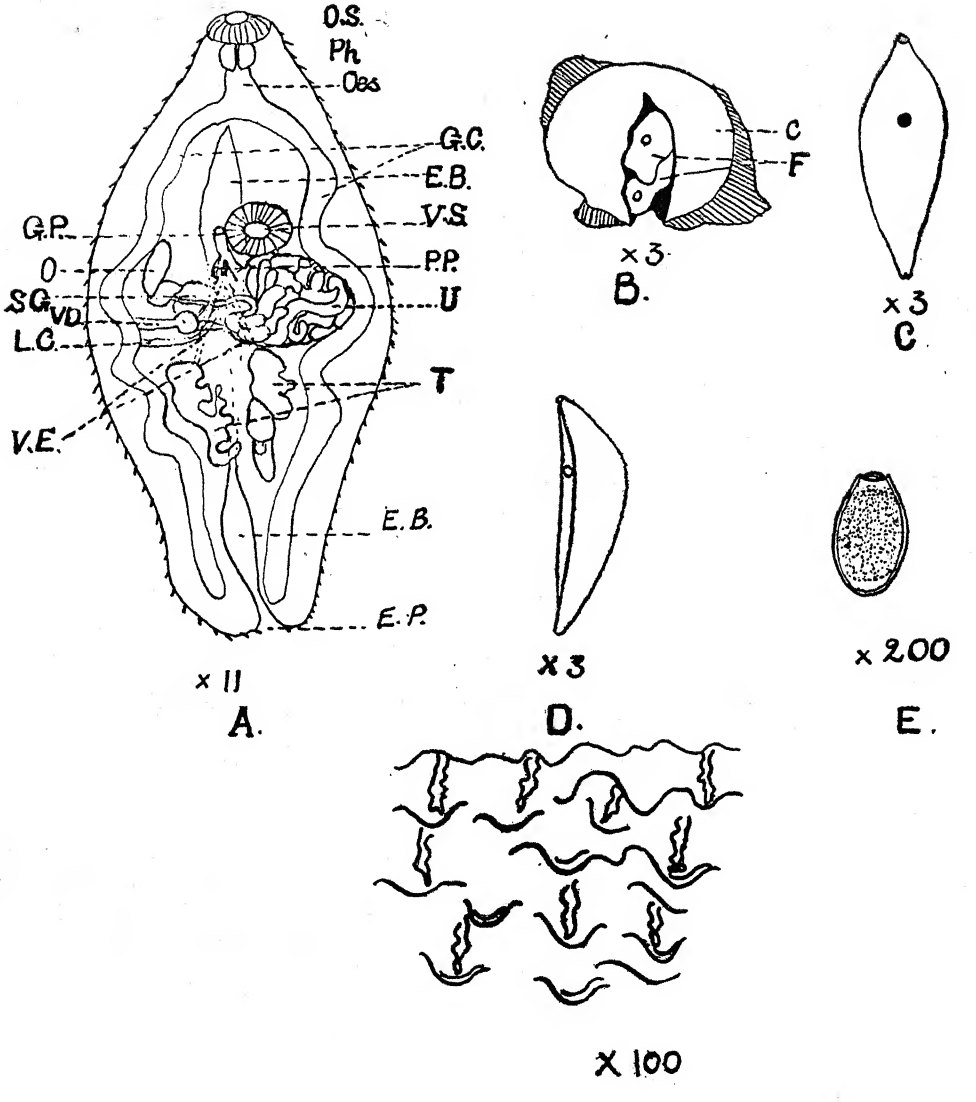
Do. D. Diagrammatic sketch of the fluke. Naked eye appearance, side view. (× 3.)

Do. E. Camera lucida drawing of an egg. (× 200.)

Do. F. Camera lucida drawing of cuticular spines. (× 100.)

ABBREVIATIONS USED.

C.	cyst.
E. B.	excretory bladder.
E. P.	excretory pore.
F.	flukes.
G. C.	gut caeca.
G. P.	genital pore.
L. C.	Laurer's canal.
O.	ovary.
Oes.	oesophagus.
O. S.	oral sucker.
Ph.	pharynx.
P. P.	pars prostatica.
S. G.	shell gland.
T.	testes.
V. E.	vasa efferentia.
V. S.	ventral sucker.


O.S.
Ph.
Oes.
G.C.
E.B.
V.S.
G.P.
P.P.
O.
U
SG
VD
L.C.
T
V.E.
E.B.
E.P.
x 11
A.
C
F
x 3
B.
x 3
C
x 3
D.
x 200
E.
X 100
F

bifurcates and the two cæca extend in a zigzag manner a short way in front of the posterior tip.

Excretory system. The excretory bladder, which opens to the exterior by a very small notch at the posterior extremity, is an enormous vesicle, extends up to the point where the gut bifurcates and forms as it were an axis round which other organs are grouped. It is provided with small excretory ducts from all over the body.

Genital system. The ovary is a single, and two-lobed structure, and lies usually towards the right, in front of the middle of the body. The recepticulum seminis meets the Laurer's canal near the termination of the oviduct, near which the common vitelline duct and the shell gland pour their contents. The uterus is a compact mass bounded anteriorly by the ventral sucker, and the transverse vitelline duct posteriorly, though in some cases it partly overlaps the latter. Vitellaria extend all over except the dorsal and ventral middle lines. Eggs are large, thin shelled and measure 60 to 100 microns in length by 35 to 50 microns in breadth. Testes are paired and lobed. The left testicle appears to project out slightly posterior to the right testicle, probably because of the pressure of the massive uterus on that side. Vasa efferentia run dorsally and meet to form a vas deferens which ends in pars prostatica. The latter leads into a genital sinus which opens to the exterior always close to the right border of the acetabulum.

Systematic position. By virtue of the possession of the above-mentioned characters these flukes fall under the family *Troglotremidae*, Odhner, 1914, and are referable to the genus *Paragonimus*.

The species compares as follows with *P. westermanii* (Kerbert, 1875) from tiger, man, cat, and pig, and *P. rudis* and *P. compactus* from a Brazilian otter and an Indian ichneumon respectively :—

——	*P. westermanii*	*P. rudis*	*P. compactus*	*P. edwardsi*, n. sp.
1. Body . .	Oval. (See figure for *P. ringeri* in Fantham, Stephens and Theobald.	Elliptical.	Ovate or oblong.	Spindle shaped.
2. Cuticular spines .	Vary in size, largest laterally.	....	Spines arranged in clusters.	Spines of the same size all over the body, arranged singly each spine with an irregular outline.
3. Oral sucker .	Ventro-subterminal.	Terminal, circular.	Terminal, orbicular.	Terminal, circular.
4. Ventral sucker .	Somewhat in front of the middle of the body, slightly bigger than the oral sucker.	As big as oral sucker, antero-subcentral, circular aperture.	Subcentral, triangular aperture, same size as the oral sucker, anterior of the middle of the body.	Bigger than the oral sucker situated at $\frac{1}{3}$rd of the whole length from the anterior end, circular aperture.
5. Genital pore .	Posterior to or left of ventral sucker.	Caudad of acetabulum.	immediately below or a little to left of ventral sucker.	Just near the right border of acetabulum.
6. Prebulbar oesophagus.	Absent.	....	Absent in Cobbold's diagram.	Absent.

—	*P. westermanii.*	*P. rudies.*	*P. compactus.*	*P. edwardsi, n. sp.*
7. Pharynx . .	Sharply defined.	....	Appears defined in Cobbold's diagram.	Sharply defined.
8. Postbulbar portion.	Delicate.	....	....	Short and delicate.
9. Caeca . .	Angularly curving.	....	Not much curved in diagram.	Lie in a zigzag way.
10. Testes . .	Lobed, both in same level.	....	Globular, one behind the other.	Slightly lobed, appear more so in over compressed specimens.
11. Ovary . .	Dichotomously branched.	....	....	Two lobes only.
12. Vitellaria . .	Underlie the whole surface posterior of the fork.	....	Extend from the anterior to the posterior extremity.	Extend from right behind the oral sucker to the posterior end.
13. Eggs . .	Oval, with indistinct operculum.	....	....	Distinct operculum but appears indistinct in degenerated eggs.

The writer has pleasure in dedicating this species to Mr. J. T. Edwards, Director, Imperial Institute of Veterinary Research, Muktesar, India. He also takes this opportunity of thanking Messrs. H. Cooper and S. K. Sen for their valuable help.

References.

Ward and Hirsch (1915). The Species of *Paragonimus* and their differentiation. *Ann. Trop. Med. and Parasit.*, Vol. IX, pp. 109-152.

Stiles and Hassal (1899). Notes on Parasites. *Sixteenth Annual Report, Bureau of Animal Industry, Department of Agriculture*, pp. 559-611.

Cobbold (1864). *Entozoa*, p. 16.

Fantham, Stephens, and Theobald (1916). *Animal Parasites of Man*, pp. 249-251.

Lane and Low (1922). In Byam and Archibald, *The Practice of Medicine in the Tropics*, Vol. III, pp. 1788-1794.

Surveyor (1919). Case of Distoma Disease in India. *Ind. Jl. Med. Res.*, (Special Indian Science Congress Number 1919), pp. 214-216.

Vevers (1923). Observations on the genus *Paragonimus* Braun, with a Redescription of *P. Compactus* (Cobbold, 1859) 1899. *H. Helminthology*, Vol. I, No. 1, p. 9220. [Abst. *Trop. Vet. Bull.* Vol. II, No. 4, p. 129.]

November 1926 Veterinary Series Vol. III, No. 9

Memoirs of the Department of Agriculture in India

On the Occurrence of Isospora and Balantidium in Cattle

BY

HUGH COOPER, M.R.C.V.S., and AMARNATH GULATI, M.Sc. (Punjab)

Imperial Institute of Veterinary Research, Muktesar

AGRICULTURAL RESEARCH INSTITUTE, PUSA

Calcutta: Government of India
Central Publication Branch
1926

ON THE OCCURRENCE OF *ISOSPORA* AND *BALANTIDIUM* IN CATTLE

BY

HUGH COOPER, M.R.C.V.S., AND AMARNATH GULATI, M.Sc., (PUNJAB),

Imperial Institute of Veterinary Research, Muktesar.

(*Received for publication on 2nd March 1926.*)

In the course of a tour in the Province of Assam during the winter of 1924-25, a systematic examination for coccidia was conducted upon samples of faeces obtained from animals. Among the animals examined were 181 cattle, and in the faeces of five of them oocysts belonging to *Isospora* were detected. No previous reference to coccidia of cattle belonging to this genus occurs in the available literature.

In each of the samples these oocysts were extremely rare, and only in one animal were they detected in a second sample obtained for the purpose of confirmation and further study. In two samples oocysts were also found belonging to *Eimeria* and one of these samples again was subsequently found to contain in addition infusoria of the genus *Balantidium*. As in the case of *Isospora*, the genus *Balantidium* does not appear to have been previously recorded from cattle.

It was not possible to take measurements or to make a detailed study of the coccidia at the time of their discovery, and the samples of faeces containing them were accordingly preserved in formalin solution with a view to submitting them to a detailed examination subsequently at the laboratory. Preservation in this way had been found to be quite satisfactory in the case of *Eimeria* of cattle, but an interval of some eight months having unavoidably elapsed between collection and examination in this case, it was found that not a single oocyst could be recognised.

The oocysts of *Isospora*, however, differed from those of *Eimeria* of cattle in that they were quite round in outline and sporogonous development took place much more rapidly, for within twenty-four hours two fully formed sporoblasts were in all cases seen. The routine method of examination employed in the work was that described by Sheather, but after centrifugation the tubes were allowed to stand for a whole day before smear preparations were made from them.

Results of examination of all preserved samples of faeces together with particulars of the animals are shown in Table I. For the purpose of detailed examination both direct smear and concentration methods were employed.

TABLE I.

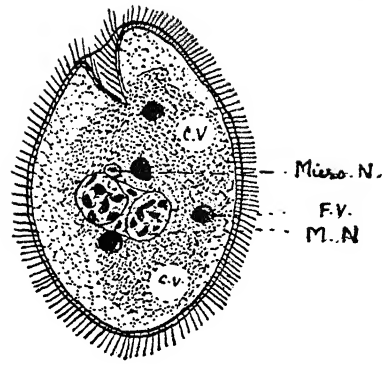
Examination of preserved samples of faeces with particulars of the animals.

Animal	Age	Place	Date of collection of samples	Parasites detected		
				Isospora	Eimeria	Balantidium
Heifer calf	15 or 16 days	Dhupatal	16-11-24	several oocysts	*Nil.*	*Nil.*
	..	..	20-11-24	*Nil.*	*Nil.*	Not examined.
	..	..	12-12-24	*Nil.*	*Nil.*	Not examined.
Bull calf	6 months	Tocklai	17-12-24	One oocyst	*Nil.*	Not examined.
Cow	2nd calf	Tocklai	6-1-25	One oocyst	*Nil.*	*Nil.*
	..	..	7-1-25	Two oocysts	*Nil.*	*Nil.*
Cow	Aged	Tocklai	6-1-25	One oocyst	Three oocysts.	Not examined.
Heifer	2½ years	Shillong	22-2-25	One oocyst	14 oocysts.	Over 60 specimens in about two grams.
	..	..	23-2-25	*Nil.*	*Nil.*	Not examined.
	..	..	24-2-25	*Nil.*	*Nil.*	Not examined.

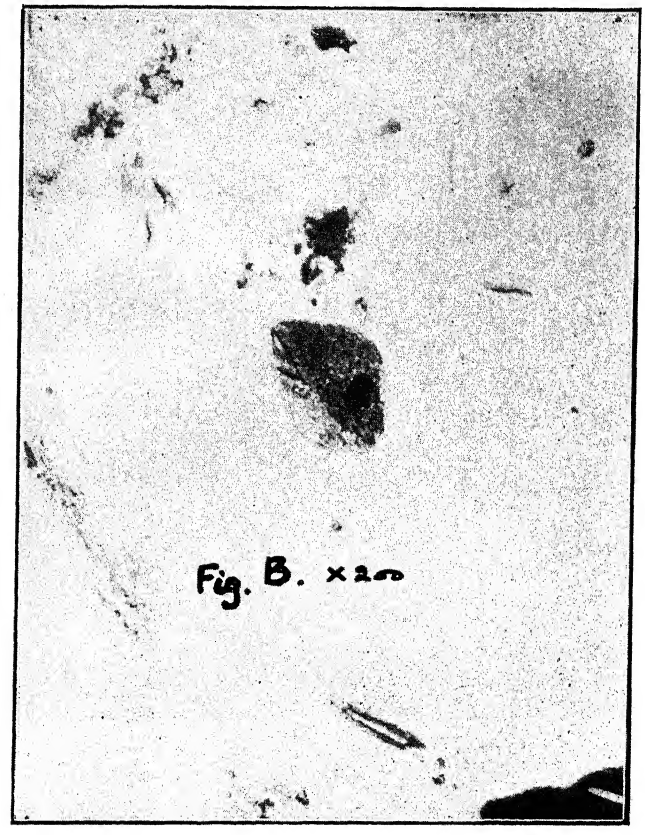
The species of *Balantidium* referred to above was detected in direct smears, the infusoria being characterized as follows :—

The body is more or less egg shaped, narrower and tapering anteriorly, broad and rounded posteriorly. Peristome short and funnel-like, inclined obliquely towards the median plane. As in the case of *Balantidium coli* of man peristome in this species also is situated near the anterior pole, and is not quite terminal. Length and breadth are in the ratio of 4 : 3. Greatest breadth is in the middle of the body. Measurements of twenty-five specimens showed a wide range of variation of size, the largest specimen being double the size of the smallest specimen. Length, 60-120 microns ; breadth 44-90 microns. Cell protoplasm divisible into a clear cortical ectoplasm and a medullary densely granular endoplasm ; outline of the latter rather irregular and by no means concentric to the body contour. The ectoplasm can be further differentiated into three layers situated in the following sequence :—

1. The pellicle or outermost covering of the organism is a thick and tough membrane through which the cilia effect their passage, the only permanent pore perforating it being the mouth.
2. A layer of basal granules from which the cilia originate. These granules are situated very close to each other so that they appear to form a continuous layer.
3. The ectoplasm below the layer of basal granules can only be faintly differentiated. It appears to be clearer and less granular than the endoplasm.



Diagrammatic sketch of *Balantidium Coli* var. bovis ×400.
(C. V. = contractile vacuole ; F.V = Food vacuole ; M. N. = Macronucleus ; Micro, N. = Micronucleus.



Photograph of *Balantidium Coli* var. bovis ×200

The endoplasm is densely granular and contains food vacuoles irregularly scattered throughout. The macronucleus is a ribbon-like structure folded at each end, appearing oval or bean-shaped in a darkly stained specimen, in which case folds at the two ends cannot be seen. Internally it shows a large number of scattered chromatin particles. The micronucleus always lies close to the macronucleus and possesses a distinct nuclear membrane and a rounded or slightly elongated karyosome placed centrally, with a clear achromatic halo around it.

The cilia are of two distinct sizes. The entire body is covered with fine, small and closely set cilia, arranged in longitudinal parallel rows. They are all of uniform length except those in the region of the peristome which are appreciably longer. They arise from basal granules, the structure of which cannot be made out on account of their being closely set and they appear to form a continuous layer below the pellicle, as described above.

Only two vacuoles can be interpreted as contractile vacuoles, and only in one specimen was a structure noticeable leading out from a vacuole, which appeared to be comparable to a cytopyge.

This species of *Balantidium*, entozoic in a heifer, as described above, resembles *B. coli* of man, except in the following particulars :—

1. The greatest breadth is in the middle, whereas in the case of *B. coli* it is posterior to the middle.

2. The macronucleus is not oval as in *B. coli*, but is ribbon like with folded ends.

In view of these differences, and on account of its occurrence in a new host (cattle), we propose the name *Balantidium coli* var. *bovis* for this species.

References.

Sheather, A. L. 1923. *Jl. Comp. Path. & Therap*, Vol. **36**, No. 2, pp. 71-90.

Hegner & Taliaferro. 1924. *Human Protozoology.* Macmillan, New York.

Minchin, E. A. 1912. *An Introduction to the Study of the Protozoa.* Arnold, London.

Strong, R. P. 1904. The Clinical and Pathological Significance of *Balantidium coli*. *Publications of the Bureau of Government Laboratories* (*Biological Laboratory*), Manilla, No. 26.

Bezzenberger, E. 1904. Ueber Infusorien aus Asiatichen Anuren. *Arch. f. Protisten.* Band 111, p. 138.

MGIPC—M—IV-2-31—3-1-27—600.